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RELATION OF CARDIAC LESIONS TO THE CLINICAL COURSE OF RHEUMATIC FEVER

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Most investigators are agreed that observed differences in the frequency of the myocardial Aschoff body in rheumatic heart disease are the result of the transient nature of the lesion. Aschoff [1] recognized this relation, and others [2] have observed the disappearance of the Aschoff body after the disappearance of symptoms of rheumatic fever.

More recently, Gross and Ehrlich [3] have described the development of the Aschoff body and have attempted to correlate the changes with the clinical course of the disease. They found that the lesion was common in cases in which the infection showed "clinical or anatomical evidence of activity" but infrequent when the disease pursued a chronic course.

At the present time the Aschoff body is almost universally accepted as evidence of existing rheumatic disease, but the terms active infection and activity are ill defined. Active infection has been assumed to occur with changes varying from leukocytosis or persistent tachycardia to typical migratory polyarthritis and with lesions as diverse as scattered groups of lymphocytes in a valve leaflet and typical Aschoff bodies.

From 2,300 consecutive cases in which autopsy was done at the New York Hospital were selected all those in which there was, on the one hand, clinical evidence of rheumatic fever, that is, a definite history of migratory polyarthritis, chorea or pains about joints, sometimes designated as growing pains, or, on the other hand, verrucae, acute pericarditis or chronic endocarditis. Cases were included in the series only if one or more of these lesions were present. Ninety-eight cases

From the Department of Pathology of Cornell University Medical College and the New York Hospital.

1. Aschoff, L.: Zur Myocarditisfrage, Verhandl. d. deutsch. path. Gesellsch. **8**:46, 1904.

2. Talalajew, W. T.: Der akute Rheumatismus, Klin. Wchnschr. **8**:124, 1929. Klinge, F.: Der Rheumatismus, Ergebn. d. allg. Path. u. path. Anat. **27**:1, 1933.

3. Gross, L., and Ehrlich, J. C.: Studies on the Myocardial Aschoff Body, Am. J. Path. **10**:467 and 489, 1934.

fulfilled this criterion. Aschoff bodies were accepted as unequivocal evidence of the disease, and no case in which these lesions were present was excluded, even if a history of the named symptoms could not be obtained. In 3 cases in which acute or chronic endocarditis was evident Aschoff bodies were found, although there was no history of rheumatic fever. These cases will be considered separately.

The number of heart sections examined for microscopic lesions varied in different cases from 2 to 6, with an average of 3 sections per case.

Of the 98 cases in which both clinical evidence of rheumatic fever and lesions of the endocardium or the pericardium were present, there were Aschoff bodies in the myocardium in 28 (28.5 per cent). The cases fell into two groups, depending on whether an attack of polyarthritis had occurred within five months of the time of death. There was a record of chorea in 9 cases, and the interval between an attack and death varied between one and a half and thirty-six years. Chorea was not included in this distinction, because no deaths occurred within five months of an attack. Vague joint and growing pains were not included because these symptoms were frequently so indefinite that time relations could not be determined. The following tabulation indicates the results of such grouping:

Interval Between Last Attack of Polyarthritis and Death	Total No. of Cases	Occurence of Aschoff Bodies	
		No. of Cases	Percentage of Cases
5 months or less	27	27	100
More than 5 months	71	1	1.4
Total	98	28	28.5

In cases in which Aschoff bodies were found the longest period that had elapsed between the last attack of polyarthritis and death was five months. This period was four and five months, respectively, in only 2 cases, and in the case in which the interval was five months some of the Aschoff bodies were recognized with difficulty because they were masked by interstitial fibrosis and in places completely obliterated. Similar fibrosis with no recognizable Aschoff bodies was observed in many of the sections from hearts of patients who died more than five months after the last attack of polyarthritis.

In 1 case in which Aschoff bodies were present the interval between the last attack of polyarthritis and death was more than five months. The patient in this case was a 57 year old man who at autopsy showed advanced mitral stenosis and fresh verrucae. The last attack of polyarthritis occurred at the age of 20 years. His heart began to decompensate at the age of 45, and at this time he complained of fleeting joint

pains, which continued until death. Five months before death he was seen in the cardiac clinic, at which time he complained of joint pains and "pains all over the body."

In 3 cases in which Aschoff bodies were recognized the patients gave no history of polyarthritis, chorea, joint pains or growing pains at any time. The first patient was a 23 year old man who at autopsy showed advanced mitral and aortic stenosis, fresh verrucae and fresh pericarditis. He gave a history of frequently repeated sore throat with fever but no joint symptoms or chorea. He died of cardiac failure two weeks after he contracted pharyngitis caused by a beta hemolytic streptococcus.

The second patient was a 25 year old man with mitral stenosis and fresh verrucae recognized at autopsy. He gave no history of arthritis or chorea and died of lobar pneumonia.

The third patient was a 25 year old woman who had fresh verrucae but no evidence of chronic endocarditis. She died of fulminant bronchopneumonia caused by a hemolytic streptococcus and meningoencephalitis.

Of 31 cases in which Aschoff bodies were recognized, an attack of polyarthritis had occurred in 27 within five months before death, vague joint pains in 1 and sore throat in 1, and in 2 no symptoms suggestive of rheumatic infection had been recorded. Aschoff bodies were found in only 4 (5.4 per cent) of 74 cases in which there was evidence of endocarditis or pericarditis but no history of recent polyarthritis.

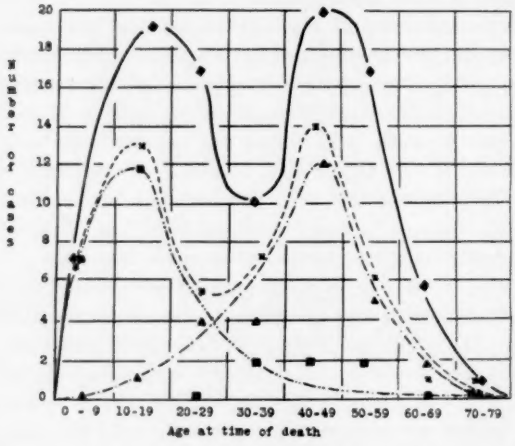
A part of this series of cases had previously been studied by Delavett,[4] who divided them into groups according to the age of the patient at the time of death. He found that of those cases in which there was evidence of endocarditis and a history of rheumatic fever Aschoff bodies were present in the myocardium in 87 per cent in which death occurred between the ages of a few months and 9 years, in 63 per cent in which death occurred between the ages of 10 and 19 years and in only 10 per cent in which death occurred after the age of 20. The following tabulation shows this age distribution in the present series.

		Occurrence of Aschoff Bodies	
Age, Yr.	No. of Cases	No. of Cases	Percentage of Cases
0-9	7	7	100
10-19	19	12	64
20-29	17	1	6
30-39	11	2	18
40-49	20	2	10
50-59	17	2	11
60-69	6	1	17
70-79	1	0	0

4. Delavett, A.: Unpublished data.

In the first decade of life Aschoff bodies were found in all cases of endocarditis or pericarditis in which there was a history of rheumatic fever. In the second decade Aschoff bodies were present in 64 per cent of cases but in only 11 per cent of cases in later decades.

Fresh verrucae and fibrinous pericarditis have been accepted by many investigators as anatomic evidence of active rheumatic infection. In this series, of 27 cases in which recent attacks of polyarthritis had occurred, Aschoff bodies were present in all cases, verrucae in 89 per cent and pericarditis in 60 per cent. Of 71 cases in which there was no record of a recent attack of polyarthritis, Aschoff bodies occurred in 1.4 per cent, verrucae in 5.5 per cent and pericarditis in 4.5 per cent. No con-



The distribution, according to age, of cardiac failure and the occurrence of Aschoff bodies in 98 cases in which there were both clinical symptoms of rheumatic fever and evidence either of endocarditis or of pericarditis at autopsy. The symbols bear the following significance: diamond, total number of deaths from all causes; cross, total number of deaths from cardiac failure; square, number of deaths from cardiac failure with Aschoff bodies recognized at autopsy, and triangle, number of deaths from cardiac failure without recognition of Aschoff bodies at autopsy.

stant relation between the clinical symptoms and these different combinations of lesions could be found.

The series of 98 cases in which both clinical symptoms of rheumatic fever and endocarditis or pericarditis were present was analyzed on the basis of age and the cause of death. The following tabulation shows the distribution of cases according to the cause of death and the occurrence of Aschoff bodies, and the figure gives the distribution in relation

to age at the time of death and the incidence of cardiac failure and Aschoff bodies:

Cause of Death	No. of Cases	Percentage of Cases
Cardiac failure (total)	55	56
Cardiac failure with Aschoff bodies	25	26
Cardiac failure without Aschoff bodies	30	31
Subacute bacterial endocarditis	18	19
Other causes	25	26

The bimodal form of the curve (figure) representing deaths from cardiac failure is obviously dependent on the curves representing deaths from cardiac failure with Aschoff bodies, on the one hand, and without Aschoff bodies, on the other, rather than on the chance distribution of deaths due to other causes. Death from cardiac failure is apparently of two types, affecting two groups of persons which are in great part widely separated with respect to age, though they show a slight tendency to overlap. Those cases in which Aschoff bodies occur fall in the first three decades and those in which these lesions are absent fall chiefly in the age group of 40 to 60 years. It is noteworthy that since the presence of Aschoff bodies agrees to within 90 per cent with the presence of polyarthritis, the essential form of these curves would not be altered if one or the other of these criteria were used.

The lesions of the mitral valve in the 98 cases in this series were divided into four grades, depending on the degree of damage to the valves. In grade I were placed valves which showed scant thickening or verrucae without evidence of thickening. In grade II were included all valves with moderate thickening but no evidence of stenosis. Grades III and IV comprised definitely stenotic valves, the former including thickened rigid valves and the latter the more advanced lesions, which were the so-called "fish-mouth," or "button-hole," valves. The same type of arbitrary classification was applied to lesions of the aortic valve, and for each case the maximum damage to the aortic or the mitral valve was recorded (the mitral valve was damaged in over 85 per cent of cases).

The following tabulation shows by decades the distribution of different grades of valvular damage:

Age, Yr.	Grade of Valvular Damage			
	I	II	III	IV
0-9	4	2	1	0
10-19	3	13	3	0
20-29	3	4	5	6
30-39	1	2	2	5
40-49	1	3	6	10
50-59	1	2	10	3
60-69	1	2	4	0
70-79	1	0	0	0
Total number of cases	15	28	31	24

The minor grades of valvular deformity (I and II) preponderate in the first three decades. The advanced degrees of deformity tend to occur later in life, and lesions of grade IV do not appear during the first two decades.

The following tabulation shows the relation between cause of death and the degree of valvular damage:

	Grade of Valvular Damage							
	I		II		III		IV	
Cause of Death	No. of Cases	% of Cases	No. of Cases	% of Cases	No. of Cases	% of Cases	No. of Cases	% of Cases
Cardiac failure with Aschoff bodies	10	66	10	34	6	20	0	0
Cardiac failure with no Aschoff bodies	1	7	2	8	9	28	19	78
Other causes	4	27	16	58	16	52	5	22
Total number of cases	15		28		31		24	

On the one hand, the incidence of deaths from cardiac failure with Aschoff bodies in the myocardium decreases as valvular deformity increases. On the other hand, the incidence of deaths from cardiac failure without Aschoff bodies increases as deformity increases. Expressed differently, in slightly more than three fourths of all cases in which death was due to cardiac failure and in which Aschoff bodies were present the lesions of the valves were of the least deforming types (grades I and II), while in an even greater proportion of cases in which the death was due to cardiac failure and there was no evidence of Aschoff bodies the greatest deformity of the valves was evident (grades III and IV).

With the exception of a small number of cases in which the patients complained of vague joint pains or mild chorea continuing over a period of months or, in some cases, years, repeated attacks of polyarthritis were separated by quiescent periods free from symptoms, and the cases could be divided into groups depending on the number of these attacks. The group of cases in which joint pains had been noticeable over a period of years or in which chorea had been continuous are designated as continuous. The following tabulation shows the relation between the number of attacks of symptoms and the severity of valvular damage:

	Total	Grade of Deformity, No. of Cases				Deformity of Grade I or II	Deformity of Grade III or IV,
No. of Attacks	No. of Cases	I	II	III	IV	Percentage of Cases	Percentage of Cases
1	57	10	16	17	14	45	55
2	20	4	5	7	4	45	55
3 to 7	11	1	5	3	2	55	45
Continuous	10	0	2	4	4	20	80

There is no significant correlation between the number of attacks and the degree of valvular deformity except in the small group of cases in which symptoms were "continuous."

The following tabulation shows the frequency of different grades of valvular lesions when the cases are divided according to the length of the interval between the onset of symptoms and the time of death:

Interval Between Onset of Symptoms and Death, Yr.	Total No. of Cases	Grade of Deformity, No. of Cases I	II	III	IV	Deformity of Grade I or II, Percentage of Cases	Deformity of Grade III or IV, Percentage of Cases
0-4	14	8	4	2	0	85	15
5-9	14	2	9	3	0	78	22
10-19	27	1	6	10	10	24	76
20-29	21	2	4	6	9	28	72
30 +	22	2	3	11	6	22	78

It is noteworthy that when less than ten years has elapsed between onset and death, the valves are usually not stenosed (grades I and II) and the most advanced lesions (grade IV) do not occur. After ten years, however, the incidence of stenotic valves is much increased.

The following tabulation shows that the duration of the disease is more significant in determining the degree of valvular deformity than the number of attacks or the age at which onset occurs:

	Grade of Valvular Deformity			
	I	II	III	IV
Mean number of attacks.....	1.60	1.72	1.74	1.40
Mean age at onset, yr.....	17.2	13.6	15.1	14.1
Mean age at death, yr.....	27.3	28.8	39.3	37.8
Mean duration of disease, yr.....	11.1	15.2	24.2	23.7

COMMENT

Though exact definition of the relation between the Aschoff body and the clinical picture of rheumatic fever is not possible at this time, there is evident correlation between age, symptoms and Aschoff bodies. Although polyarthritis preceding death by less than five months was found at autopsy to be invariably accompanied by Aschoff bodies, these lesions were not always preceded by manifest polyarthritis, and in a few cases the existence of rheumatic infection was first ascertained on autopsy.

The course of rheumatic heart disease has two fairly distinct stages, approximately separated by the third decade of life. Cardiac failure is associated in the first stage with the presence of Aschoff bodies in the myocardium and in the second with advanced valvular deformity. In the first three decades of life symptoms of rheumatic fever and Aschoff

bodies are relatively common and are accompanied by minimal valvular deformity. Under such circumstances cardiac failure must be attributed to the myocardial involvement, since cardiac failure with minor degrees of deformity and absence of Aschoff bodies occurs only rarely. In the later decades, when cardiac failure can be attributed to advanced valvular deformity, Aschoff bodies are seldom found and have scant, if any, part in the production of cardiac failure.

Rothschild, Kugel and Gross [5] attributed cardiac failure in the first five decades of life to injury of the myocardium, as shown by the presence of Aschoff bodies, rather than to a mechanical defect produced by a valvular lesion. Swift and McEwen [6] stated that cardiac failure does not occur in young persons with rheumatic fever, even when valves are deformed, unless there is accompanying disease of the myocardium. The observations described here confirm these statements in their application to young persons but indicate that cardiac failure in later life is the result of valvular lesions.

Since the degree of valvular deformity appears to determine the appearance of cardiac failure in the later decades, the factors which influence the degree of deformity are important. Wilson [7] asserted that there is apparently a direct relation between the degree of cardiac damage and the number and severity of recurrences. Injury to the heart, she stated, is frequently insidious and subclinical, progressing with time even in the absence of manifestations of active infection. In the cases that are discussed here it is noteworthy that the only factor that has been found to increase the severity of valvular deformity is the duration of the disease.

SUMMARY

Of 98 cases in which endocarditis or pericarditis, found at autopsy, had been preceded by a history of rheumatic fever with polyarthritis or other similar manifestations of disease, Aschoff bodies were found in all in which death occurred during the first decade of life, in 64 per cent of those in which it occurred during the second decade and in 11 per cent of those in which it occurred during a later decade.

When Aschoff bodies were found in the myocardium (28 cases) with 1 exception the interval between the last attack of polyarthritis and death was five months or less.

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5. Rothschild, M. A.; Kugel, M. A., and Gross, L.: The Incidence and Significance of Active Infection in Cases of Rheumatic Cardiovascular Disease in the Various Age Periods, Am. Heart J. **9**:586, 1934.

6. Swift, H. F., and McEwen, C.: Rheumatic Fever, in Christian, H. A.: Oxford Medicine, New York, Oxford University Press, 1938, vol. 5, pt. 1, pp. 11-38.

7. Wilson, M. G.: Rheumatic Fever, New York, Commonwealth Fund, Division of Publications, 1940.

In 3 cases in which Aschoff bodies occurred in the myocardium there was no history of polyarthritis, chorea or other clinical evidence of acute rheumatic infection.

A curve of the frequency of death in cases in which the symptoms of rheumatic fever and cardiac lesions occurred at different age periods shows two distinct peaks, one in the first decades of life, corresponding with deaths from cardiac failure and the presence of Aschoff bodies in the myocardium, and the other between the ages of 40 and 60 years, associated with cardiac failure, deforming lesions of the valves and an absence of Aschoff bodies.

Minor degrees of valvular deformity preponderated in the first three decades of life, whereas advanced deformity was common in the later decades. Valvular deformity increased with the duration of the disease after the onset of symptoms but had no constant relation to the number of attacks or to the age at onset.

PULMONOCARDIAC FAILURE AS A RESULT OF SPINAL DEFORMITY

REPORT OF FIVE CASES

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An extreme deformity of the spine is generally acknowledged to be a handicap both in the physical and in the social life of the person afflicted. That the resulting distortion of the thoracic organs may have fatal consequences has not been widely recognized in the English-speaking world. For many years continental writers, even in textbooks, have described this syndrome, which has been termed "pulmonocardiac failure."

Chapman, Dill and Graybiel,[1] of the Harvard Fatigue Laboratory, recently reviewed the subject of pulmonocardiac failure and were able to find 126 fatal cases recorded in the literature. They reported the clinical and laboratory data in 12 cases of their own, in 4 of which the patients died. In their investigation they paid particular attention to the ill effects on respiratory function.

My interest in this subject was aroused by encountering 2 instances of this condition within a period of two months during the course of routine autopsies. Search of the files in this department back as far as the beginning of 1925 revealed 3 other instances among 6,700 autopsies. An analysis of the clinical and postmortem observations in these 5 cases form the basis for this report.

REPORT OF CASES

CASE 1.—H. C., a 75 year old man, was admitted to the Toronto General Hospital, service of Prof. Duncan Graham, on Oct. 19, 1933. He gave a history of slight dyspnea on exertion during the previous year but had been able to carry on his usual job as a cleaner in a laundry without real difficulty. Three weeks before admission he noticed an unusual degree of shortness of breath after climbing a flight of stairs. A week later, while working, he suddenly became short of breath and weak and had to rest frequently. In the evening of the same day his feet began to swell, but the swelling had disappeared by the following morning

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1. Chapman, E. M.; Dill, D. B., and Graybiel, A.: The Decrease in Functional Capacity of the Lungs and Heart Resulting from Deformities of the Chest: Pulmonocardiac Failure, Medicine **18**:167 (May) 1939.

and did not recur. He was forced to give up work, as the slightest exertion caused him severe respiratory distress. He was confined to his room but did not go to bed prior to admission.

On examination the patient was very dyspneic and showed marked cyanosis of the face. There was a severe deformity of the chest due to an extreme left-sided scoliosis involving most of the thoracic portion of the spine. He stated that he had had this deformity as long as he could remember, and no history of its onset or progress could be obtained. The heart was thought to be normal in size, and there were no murmurs. The blood pressure was 120 systolic and 80 diastolic, and the pulse was regular, with a rate of 74 per minute. Respirations were 30 per minute. Numerous rales were heard at the bases of both lungs. The edge of the liver was palpable 3 fingerbreadths below the costal margin. No pitting edema was present. There were no other significant physical findings.

Routine examination of the blood and urine revealed no important abnormalities. An electrocardiogram showed a voltage of 5 mm. with negative T waves in leads II and III and a tendency to right axis deviation.

The clinical diagnoses were chronic degenerative myocarditis and cardiac insufficiency with kyphosis of the spine. Because of the fairly rapid onset of symptoms and the abnormal T waves, cardiac infarction was also considered to be a possibility.

The patient was put to bed, and the diet was restricted in fluids and salt. A subsequent electrocardiogram showed a positive T wave in lead II, a change which was regarded as supporting the diagnosis of cardiac infarction. He continued to be dyspneic, the respirations varying between 20 and 38 per minute; the cyanosis deepened. The pulse rate never rose above 98 per minute; terminally the blood pressure fell to 98 systolic and 70 diastolic. Death occurred suddenly during the early morning of October 23, four days after admission.

Autopsy.—The body measured 150 cm. in length and weighed 135 pounds (61.2 Kg.). The face and fingers were extremely cyanosed; there was no edema of the legs. The chest was short and barrel shaped because of the spinal deformity, which consisted of a sharp, extreme curve to the left starting at the first thoracic vertebra and passing out nearly to the left axilla, the apex being formed by the seventh thoracic vertebra. From here the vertebral column passed downward and medially in an S-shaped curve to the sacrum. The cut surface of the lumbar portion of the spine was unusually soft and porous in consistency and pink in color; in the thoracic portion it was somewhat firmer in texture and had a grayish tinge. No evidence of tuberculosis was seen.

The pericardial sac contained 60 cc. of clear fluid. The heart weighed 410 Gm. The right auricle and ventricle were greatly dilated and filled with clot. The myocardium of the right ventricle measured 8 mm. in thickness and that of the left 15 mm.; no scarring was present on the cut surface. A slight degree of sclerosis of the coronary arteries was evident but much less than one might reasonably expect in so old a man; there was no stenosis of the arteries anywhere. No valvular lesions were seen, and there was no dilatation of the valve orifices. The aorta followed the course of the spine throughout and was not constricted at any point. The lungs were small, each weighing 265 Gm. They were crepitant throughout. The branches of the pulmonary artery were not dilated; many yellowish atheromatous nodules were scattered along the intima. No other lesions were noted in the lungs. Each pleural cavity contained 150 cc. of transudate. Two

hundred cubic centimeters of clear fluid was also present in the peritoneal cavity. The liver was small, weighing 1,120 Gm.; the cut surface showed the characteristics of passive congestion. There were no other gross findings of importance.

Microscopic Examination.—The sections of heart stained by hematoxylin and eosin were normal, but in those stained with sudan IV many fine fat droplets were seen dotting the muscle fibers. In the lungs there was congestion of the capillaries and a few of the alveoli contained edema fluid. Some of the alveoli were partially collapsed; the walls appeared to be of normal thickness. No significant sclerosis was noted in the small arteries or arterioles. Well developed chronic passive congestion was evident in the liver. In the thoracic vertebrae the trabeculae were scanty and rather thin but otherwise not remarkable. The marrow did not contain any lesions. None of the other organs sectioned revealed any significant abnormalities.

Diagnosis.—The principal anatomic diagnoses were osteoporosis of the spine (cause undetermined), scoliosis of the thoracic and lumbar portions of the spine, hypertrophy and dilatation of the right auricle and ventricle, fatty degeneration of the heart, atherosclerosis of the pulmonary artery, hydrothorax, ascites and passive congestion of the liver.

Case 2.—A. F., a 58 year old man, was admitted to the medical service of the Toronto General Hospital on April 16, 1935. He had always been more or less short of breath on exertion, a disability which he himself attributed to his thoracic deformity. About four months before admission his landlady had drawn his attention to the bluish color of his face and neck, which was present intermittently and was more noticeable after exercise. For the past two months there had been a definite increase both in the dyspnea and in the cyanosis. Two weeks before admission he began to have swelling of the feet and legs toward evening; this usually had disappeared by the following morning. There was no precordial pain, palpitation or cough. An extreme curvature of the spine had been present ever since birth.

On examination the patient showed severe cyanosis of the face and neck but did not appear to be in acute respiratory distress. The eyelids were slightly swollen and the scleral vessels were congested. The chest was short, narrow and deep, because of marked left-sided scoliosis of the thoracic portion of the spine with slight lordosis in the lower part. The size of the heart could not be estimated clinically; there were no murmurs. The pulse was regular, the rate being 96 per minute. The blood pressure was 120 systolic and 66 diastolic. Many moist rales were heard throughout both sides of the chest. In the right side of the chest posteriorly there was one small patch of bronchial breathing. The liver was not enlarged. Pitting edema was detected up to the level of the knees.

Routine laboratory examination failed to reveal any abnormality of urine or blood. The Wassermann reaction of the blood was negative. An electrocardiogram showed a voltage of 8 mm. and right axis deviation; the T wave in lead II was flat, and the T wave in lead III was negative. On fluoroscopic examination the heart was thought to be enlarged, but the thoracic deformity made any definite conclusion impossible. Patchy consolidation was observed throughout both lungs and was thought to be pneumonic in character.

A diagnosis of chronic degenerative myocarditis with cardiac failure and bronchopneumonia was made; because of the extreme cyanosis of the face, the presence of tumor or aneurysm in the superior part of the mediastinum was suspected.

The patient's temperature varied between 99 and 101.4 F. and his pulse between 90 and 110 per minute, never being over 120 per minute. On one occasion it was observed that the blood pressure in the right arm was 118 systolic and 78 diastolic and that in the left arm was 96 systolic and 64 diastolic. His course was progressively downhill, and he died on April 23, after one week in the hospital.

Autopsy.—The body was that of a small man with a short neck and the head sunken between the shoulders. It weighed 125 pounds (56.7 Kg.) and measured 140 cm. in length. The face and neck were deeply cyanosed, the lips being almost black. The veins of the neck were greatly distended. The eyes were bulging, the eyelids edematous and the conjunctivas much congested. Pitting edema was evident up to the midthigh level. Extreme left-sided scoliosis was present in the midthoracic region, with bulging of the left side of the chest and narrowing of the sternum anteriorly. Except for the distortion of the vertebrae, the cut surface of the spine showed no lesions.

The heart was rather large and globular and weighed 375 Gm. The pericardial sac contained 100 cc. of clear fluid. Much dark red, fluid blood welled up from the severed ends of the pulmonary vessels; the superior vena cava was distended. The right ventricle was dilated and greatly hypertrophied, its wall measuring 14 mm. in thickness, the same as that of the left ventricle. No valvular lesions were seen. The orifice of the tricuspid valve measured 11 cm. in circumference and that of the pulmonary valve 7.3 cm. The right auricular appendage was filled with a thrombus. Slicing of the myocardium failed to reveal any lesions. The coronary vessels contained many atheromatous plaques but were widely patent except at one point 2 cm. from the origin of the anterior descending branch, where the lumen was almost occluded. The aorta was small, measuring 4.5 cm. in its greatest circumference; it was closely applied to the spine in the latter's tortuous course but was not obstructed.

The right pleural cavity was occupied by 100 cc. of yellow transudate; the left was empty. The lungs were both small, the left weighing 350 Gm. and the right 310 Gm. Because of the thoracic deformity the left lung lay in an almost horizontal position. Both lungs were congested, and poorly defined areas of consolidation could be felt in the upper lobe of the left lung and the lower lobe of the right one. Elsewhere the cut surface was flabby, dark red and moist. No stenosis of the pulmonary arteries was evident, but examination of the intimal surface disclosed numerous discrete yellowish atheromatous plaques.

Six hundred cubic centimeters of ascitic fluid was removed from the peritoneal cavity; the intestine was everywhere edematous; the hemorrhoidal veins were distended and filled with thrombi. The liver weighed only 900 Gm. and was deeply congested. The rest of the abdominal organs were not remarkable except for congestion.

Microscopic Examination.—Sections of the heart muscle did not reveal any fibrosis or other lesion. In the lungs there was much congestion and many of the alveoli were partially collapsed; hemorrhage had occurred into the air spaces, which also contained macrophages laden with blood pigment. There was no thickening of alveolar walls. Some of the small branches of the pulmonary artery contained patches of intimal hyaline thickening in their walls, but there was no significant narrowing of the lumens; the arterioles appeared normal. No inflammatory changes were observed. In the liver a severe degree of chronic passive congestion was evident. The sinusoids of the central and midzonal portions of the lobules were distended with red cells, the liver cords being much compressed. No important lesions were encountered in the other organs.

Diagnosis.—The chief anatomic diagnoses were scoliosis and lordosis of the thoracic portion of the spine (congenital), hypertrophy and dilatation of the right ventricle (extreme), atherosclerosis of the pulmonary artery, passive congestion of the lungs and liver, hydrothorax (left side), hydropericardium, ascites and edema of the legs.

CASE 3.—R. L., a 49 year old man, was admitted to the medical service of the Toronto General Hospital on June 20, 1936. He was so ill that a detailed history could not be obtained. Ten years previously he had been told that he had heart disease, but he apparently had had no symptoms until three months before admission, when he began to have dyspnea, which grew progressively worse. One week before admission this became so severe that he had to remain in bed in a prone position with his head propped on his hands.

Examination revealed a hunchback in acute respiratory distress. Cyanosis was marked and breathing was labored and rapid, the respiratory rate being 60 per minute. Extreme kyphosis was present in the midthoracic portion of the spine. A history was later obtained from the brother that the patient had suffered an injury of his spine at the age of 2 years and the deformity had dated from that time. The size of the heart could not be determined; the heart sounds were faint and no murmurs were heard. The apex rate was 150 per minute and the beat was regular. The blood pressure could not be accurately determined, but the systolic pressure was less than 40 mm. of mercury. Moist rales were heard at the bases of both lungs. The edge of the liver was palpable 4 fingerbreadths below the costal margin. Pitting edema was absent.

A diagnosis of chronic degenerative myocarditis with cardiac failure was made.

One half milligram of a glucoside from Digitalis lanata (digoxin) was given intravenously, and in half an hour the pulse rate had decreased to 120 per minute; respiration dropped to 30 per minute, and the cyanosis deepened. The patient's condition became progressively worse, and in spite of the administration of caffeine and coramine (a 25 per cent solution of pyridine betacarboxylic acid diethylamide) he died two hours after admission.

Autopsy.—The body was that of a poorly nourished, hunchbacked man, measuring 144 cm. in length and weighing 85 pounds (38.6 Kg.). Cyanosis of the face, ears and buccal mucosa was prominent. Extreme kyphosis of the midthoracic portion of the spine was present, accompanied by marked pigeon breast deformity. The cut surface of the spine was normal in appearance. No dependent edema was demonstrated. The pericardial fluid amounted to only 15 cc. The heart lay farther to the left of the midline than usual, and almost the whole of its anterior surface was formed by the right ventricle, which was greatly dilated and hypertrophied; its wall was 10 mm. in thickness, while that of the left ventricle was 14 mm. The heart weighed 360 Gm. No valvular lesions were evident; the tricuspid valve measured 11.5 cm. in circumference and the pulmonary valve 7.5 cm. The coronary arteries were dotted with numerous atherosclerotic plaques, but there was no significant stenosis. The cut surface of the interventricular septum was flecked by a few small patches of fibrosis. The aorta was acutely angulated at its arch, but no important degree of obstruction was obvious.

The right pleural cavity did not contain fluid, but there was 75 cc. of clear amber fluid in the left one. The left lung weighed 327 Gm. and the right 310 Gm. Both were congested and rather moist. The intima of the pulmonary arteries was occupied by many atheromatous patches. No fluid was found in the peritoneal sac.

The liver weighed 923 Gm. and presented no abnormalities. The other organs were not remarkable except for an adenoma beneath the capsule of the right kidney and a benign ulcer on the lesser curvature of the stomach near the pylorus.

Microscopic Examination.—In the heart a few small patches of fibrosis were evident. Several of the branches of the coronary arteries showed some medial hypertrophy. Sections of the lungs revealed partial collapse of alveoli, with slight fibrous thickening of their walls and patchy congestion. A few collections of heart failure cells were seen. In the small branches of the pulmonary artery hyaline intimal plaques were visible, but these had no significant effect on the size of the lumens. The arterioles, however, had a greatly hypertrophied media, such that the lumens were on the average reduced in size by a third. The liver presented slight passive congestion around central vein areas.

CASE 4.—P. M., a 31 year old man, was admitted to the Toronto General Hospital, service of Prof. Duncan Graham, on Oct. 29, 1940. For the past ten years he had been rather tired and somewhat short of breath on exertion but not sufficiently so that the condition interfered with his work. About five months before admission he noticed a definite increase in his fatigue and an intermittent dry cough developed. One week before admission he had a "chill," followed by fever and generalized aches. During the rest of this week he had some difficulty in getting his breath and experienced increased cough, with scanty white sputum and frequent attacks of nausea and vomiting. His physician found many rales and rhonchi in his chest and sent him to the hospital with the diagnosis of "flu."

On admission the patient was rather drowsy, slightly cyanosed and dyspneic. He complained of dimness of vision, apparently of recent onset. There was well marked kyphosis of the thoracic portion of the spine with some scoliosis to the left. At the age of 5 he had had tuberculosis of the spine, resulting in this deformity, which had apparently not progressed in recent years. Examination of his eyes revealed that he could only distinguish light from dark, but no basis for this could be found. Many rhonchi and rales were heard throughout the chest. The heart seemed to be enlarged to percussion; there were no murmurs. The pulse was regular, the rate being 80 per minute. The blood pressure measured 170 systolic and 90 diastolic. The liver was not palpable, and there was no dependent edema. Neurologic examination showed exaggeration of all tendon reflexes and an exhaustible ankle clonus.

Laboratory examination revealed that the urine had a specific gravity of 1.010, with a 1 plus reaction for albumin and a few white blood cells. The Wassermann reaction of the blood was negative, and the nonprotein nitrogen content of the blood was 172 mg. per hundred cubic centimeters.

A diagnosis of hypertensive heart disease, pulmonary edema and uremia was made.

The next day the patient was more drowsy, and intermittent twitching of the left leg developed, which was followed two hours later by clonic spasm of all limbs and conjugate deviation of the eyes to the right; this attack lasted about two minutes. During this time he did not lose consciousness but became more deeply cyanosed than usual. It was noted that the cyanosis was especially marked over the head, neck and upper part of the chest; there was also much engorgement of the veins of the neck and arms. These findings rather suggested a mediastinal tumor, but roentgenologic examination of the chest did not confirm this diagnosis. Convulsions continued at irregular intervals for the next two days. An electrocardiogram showed a voltage of 7 mm. and depressed RT segments in

leads II and III. Treatment consisted of the administration of sedatives, intravenous injection of dextrose solution and use of an oxygen tent; on one occasion a venesection was performed, 400 cc. of blood being removed. The venous pressure in the left arm was 360 to 380 mm. of water and in the left leg 290 mm. On October 31 the pulse was regular, with a rate of 100 per minute, and the blood pressure was 150 systolic and 110 diastolic. The nonprotein nitrogen content of the blood had fallen to 146 mg. per hundred cubic centimeters, and the creatinine content was 6 mg. per hundred cubic centimeters. The urine contained a tract of albumin and an occasional white blood cell. On November 1 the patient became semiconscious; the level of nonprotein nitrogen rose to 154 mg. per hundred cubic centimeters and the level of creatinine to 9.4 mg. During the night of November 2 he had another convulsion, became comatose and died, four days after admission.

Autopsy.—The body measured 140 cm. in length and weighed 103 pounds (46.7 Kg.). A severe degree of kyphosis with slight scoliosis to the left side was noted in the upper and middle thoracic regions. Externally the vertebral bodies appeared normal except for their deformity. Only the lower thoracic and the lumbar portion of the spine were removed, and their cut surfaces revealed no lesions; the marrow was deep red. Cyanosis was present over the face and neck. No dependent edema was detected. The pericardial sac contained a normal amount of fluid.

The heart showed considerable dilatation of the right auricle and ventricle and weighed 250 Gm. The valves were normal and the valve rings were not enlarged. The coronary arteries were widely patent, thin walled and smooth. The right ventricular wall was somewhat hypertrophied, measuring 7 mm. in thickness, while the left measured 12 mm. The cut surface of the myocardium presented a normal appearance. The aorta followed closely the curve of the spine but aside from this distortion was not remarkable.

The left pleural cavity was much smaller than the right one, but the anteroposterior diameter of each was much increased. No fluid was found in the pleural sacs. The left lung was small and partially collapsed, weighing 280 Gm.; the right was of normal size and weighed 510 Gm. The whole of the left lung, as well as the upper lobe of the right lung, showed congestion. The cut surfaces were moist and dull red. The intima of the pulmonary arteries was normally smooth and glistening throughout. The peritoneal cavity did not contain any fluid.

The liver weighed 1,490 Gm. and the cut surface was congested. The kidneys were congested but otherwise normal.

Microscopic Examination.—No abnormality was found in the heart. Section of the left lung revealed some collapse; there was much congestion of the capillaries, and many of the alveoli were filled with red cells and edema fluid; numerous macrophages filled with blood pigment lay free in the alveolar spaces. No thickening of the alveolar walls was evident. Essentially the same picture was present in the upper lobe of the right lung and to a much less degree in the lower lobe; here a few patches of emphysema were noted. In no section did the pulmonary vessels present any abnormality. The central vein areas of the liver were occupied by collapsed stroma, in the meshes of which were caught red blood cells and globules of bile pigment. There was much destruction of the parenchymal cells in the central zones. These changes were fairly well localized to the central zone, but the remainder of the lobule also showed widening of the sinusoids. The kidneys showed only congestion. No other important lesions were noted in any organ.

Diagnosis.—The anatomic diagnosis included kyphosis and scoliosis of the thoracic portion of the spine (tuberculous?), hypertrophy and dilatation of the right auricle and ventricle and passive congestion of the lungs, liver and kidneys. No significant lesions were present in the kidneys, and the elevation of the nonprotein nitrogen and the creatinine content of the blood could not be explained on a renal basis.

CASE 5.—F. D., a 53 year old man, was admitted to the medical service of the Toronto General Hospital on Dec. 14, 1940. He gave a history of having caught a head cold two weeks before; he felt well till two days before admission, when fatigue, malaise, anorexia and dry cough developed and forced him to go to bed. The day before entering the hospital he was short of breath even at rest and felt feverish. On close questioning he reluctantly admitted that he had been getting increasingly short of breath for some years and more especially in the past four months; his ankles had recently begun to swell toward evening but were normal by morning. For six weeks he had had frequent attacks of nocturnal dyspnea, usually about 6 a. m. He also stated that his mother had died of pulmonary tuberculosis when he was 1 year old and that shortly after tuberculosis of the spine had developed.

On examination he was dyspneic and had a grayish cyanosis. There was extreme kyphosis of the dorsal portion of the spine with pectus carinatum. The only abnormalities in the lungs were diminished breath sounds at the base of the left lung and rales at the base of both lungs. The size of the heart could not be estimated. There were no murmurs. The pulse was regular, with a rate of 98 per minute; the blood pressure measured 130 systolic and 60 diastolic. The edge of the liver was palpable 3 fingerbreadths below the costal margin. Dependent edema was not present. The rectal temperature was 101 F.

Laboratory examination of the blood and urine revealed nothing significant. The Wassermann reaction of the blood was negative and the nonprotein nitrogen content was 50 mg. per hundred cubic centimeters.

Having case 4 in mind, the clinician made a diagnosis of pulmonocardiac failure, with possible bronchopneumonia.

The patient was placed in an oxygen tent and given theophylline with ethylene diamine; he showed some temporary improvement, though dyspnea and cyanosis remained. On December 30 he became much worse; cyanosis and dyspnea were extreme, and the pulse rate rose to 160 per minute. In spite of intravenous administration of digifoline and venesection, he died on December 31, after seventeen days in the hospital.

Autopsy.—The body was that of a moderately well nourished, hunchbacked man, measuring 148 cm. in length and weighing 97 pounds (44 Kg.). Extreme kyphosis was present in the lower thoracic region, the apex of the knuckling forming an angle of about 30 degrees. Anteriorly there was a well developed pigeon breast deformity of the sternum, its apex being a handbreadth above the level of the lower sternal border. Dependent edema was present only in the scrotum.

The pericardial sac contained no excess fluid. The heart weighed 375 Gm., and its chambers were not notably dilated. The valve cusps were normal and the orifices were not enlarged, the orifice of the tricuspid valve measuring 12.5 cm. in circumference and that of the pulmonary valve 9 cm. The left anterior descending coronary artery showed severe sclerosis, with calcification and stenosis throughout most of its length; the involvement of the other branches was minimal. The wall of the left ventricle measured 15 mm. in thickness and that of the right 5 mm.

The cut surface of the myocardium presented no abnormalities. The aorta followed the course of the spine and was sharply kinked just above the celiac axis, in the hollow of the kyphotic portion of the spine. A thick, firm ridge projected inward from its anterolateral surface, narrowing the lumen by about a third.

The thoracic cage was small and deformed. The left pleural sac contained a few cubic centimeters of amber fluid; the right one was empty. Both lungs were small, the left weighing 245 Gm. and the right 295 Gm. The posterior portion of each lung was somewhat bluish, doughy and collapsed; elsewhere both lungs were normally crepitant. Opening the branches of the pulmonary artery disclosed a moderate number of yellowish patches in the intima. The liver was somewhat square and weighed 1,400 Gm. No lesions were obvious on the cut surface.

Sagittal section of the spine showed the angulation to be about 4 cm. above the end of the spinal cord. It was not possible to identify the various vertebrae, since the disks had almost completely disappeared from the seventh thoracic to the fifth lumbar vertebra, which section of the spine measured only 9 cm. in total length. At the point of angulation the vertebral bodies and the spinous processes were involved in an old fibrocaseous tuberculous process. The spinal cord was not compressed.

Microscopic Examination.—Sections of heart muscle from the lateral walls of both ventricles and the septum revealed only questionable medial hypertrophy of some of the arterioles. In the lungs there was moderate congestion of the capillaries and many alveolar spaces were filled with edema fluid and a sprinkling of macrophages, some filled with brown pigment. Patchy areas of collapse alternated with areas of emphysema. There was no thickening of the alveolar walls. In only one area did the pulmonary arteries present any abnormality. Here they showed well developed endarteritis in an area of scarring infiltrated with lymphocytes, the appearance being suggestive of a healed tuberculous lesion. The veins were normal. In the liver there was some dilatation of the central portions of the sinusoids. Sections of the vertebrae revealed zones of caseous necrosis, with dead bone alternating with areas of new bone formation.

ANALYSIS OF CASES

Pulmonocardiac failure as a result of severe deformity of the spine is not often seen in routine autopsies. In this series it was present on an average of only once in every 1,340 cases, or in approximately 0.075 per cent. It has been pointed out, however, that the condition is of frequent occurrence in persons so deformed. This is confirmed in our autopsy records by the discovery of only 1 case, not reported here, in which the patient suffered from extreme deformity of the vertebrae and died of intercurrent disease.

Clinical Observations.—It has been emphasized that persons with such deformity tend to die in early adult life, the average age at death having been given as 30 years. In this small series that poor prognosis was not borne out, the average age at death being 53 years. Only 1 patient was sufficiently young to be noteworthy. The claim of Dedic[2] that patients thus deformed rarely pass the age of 40 was not substantiated.

2. Dedic, S.: La cardiopathie cyphotique, Arch. d. mal. du cœur **23**:33, 1930.

Spinal deformities from whatever cause are known to be more common in men than in women, as is also pulmonocardiac failure. In all of these cases the patients were men.

A combination of kyphosis with well marked scoliosis is usually seen, but in 2 instances of deformity in this series, 1 due to injury and in the other to tuberculosis, kyphosis alone was present; in 1 other the kyphosis was predominant, the scoliosis being of minimal degree. In both the cases in which scoliosis was the main deformity the primary curve was to the left, as it was also in the case of kyphoscoliotic curvature. This finding is contrary to the usual experience, in which right-sided scoliosis is by far the commoner.

Symptoms, sometimes of only mild degree, were usually present for several years, or even as far back as the patient could remember. However, the length of time from the onset of the more acute manifestations to death was relatively short, only several months, and rapid deterioration of the patient's condition usually took place in the last few days. Dyspnea was the major symptom in all cases. It was often present for some years, but the patient was able to carry on with little trouble until a relatively sudden exacerbation marked the onset of the final illness. One patient could obtain relief only by lying prone with his head propped on his hands. Nocturnal dyspnea was mentioned in 1 case. Four of the patients had a history of recent, intermittent swelling of the feet, but in only 1 was this symptom present on admission. Palpitation and precordial pain were not complained of in this series. The sudden fainting attacks mentioned by various authors did not occur.

Cyanosis was evident in all cases, and involved chiefly the head and neck; in 1 case this was first noticed by another person. In 2 cases this fairly localized distribution of the cyanosis, together with dilatation of the veins, suggested a mass in the superior portion of the mediastinum. Respirations were usually labored, and the rate increased to the neighborhood of 30 per minute; terminally this rate was doubled in 2 cases.

The size of the heart was difficult to determine clinically owing to the thoracic deformity, and in most cases an opinion on this point was not even expressed. Teleroentgenograms were not made. No murmurs were detected in any case. In no case was any mention of accentuation of the pulmonic second sound made; it is possible that this would have been noted if it had specifically been looked for. The pulse rate was somewhat increased but was rarely over 120 per minute except just before death. No abnormalities of rhythm were detected. In only 1 case was the blood pressure elevated, and in this case the patient probably was suffering from essential hypertension not connected causally with the pulmonocardiac failure.

In the 3 cases in which electrocardiograms were made the voltage tended to be low, varying from 5 to 8 mm. In case 1 there was a

tendency to right axis deviation with negative T waves in leads II and III, those in the latter being cove plane in type. The T wave in lead II became positive three days later, then reverted to negative the following day. This was thought to be indicative of cardiac infarction, but the diagnosis was not confirmed at autopsy. These changes are probably more suggestive of relatively acute right ventricular strain (Barnes [3]); it will be remembered that in this case the onset of acute symptoms occurred less than a month before death. However, the thickness of the wall of the right ventricle is evidence of chronic right ventricular strain as well. In case 2 there was right axis deviation, the T wave in lead II was flat and the T wave in lead III was negative. These changes are regarded as among those characteristic of right

Pathologic Data in Five Cases of Pulmonocardiac Failure Resulting from Spinal Deformity

Case No.	Age, Yr.	Sex	Spinal Deformity	Cause of Spinal Lesion	Weight of Heart, Gm.	Dilatation of Heart	Thickness of Wall of Right Ventricle, Mm.	Combined Weight of Lungs, Gm.	Pulmonary Atherosclerosis
1	75	♂	Scoliosis (left)	Osteoporosis	410	Right auricle and ventricle	8	530	+++
2	58	♂	Scoliosis (left) with slight lordosis	Congenital	375	Right ventricle	14	660	+++
3	49	♂	Kyphosis	Injury	360	Right ventricle	10	637	+++
4	31	♂	Kyphosis with slight scoliosis (left)	Tuberculosis (?)	250	Right auricle and ventricle	7	790	0
5	53	♂	Kyphosis	Tuberculosis	375	None	5	540	++

ventricular strain.[3] It is noteworthy that this was the case in which the wall of the right ventricle measured 14 mm. in thickness. In case 4 there was only slight depression of the RT segments in leads II and III.

The only signs of congestive failure consistently present were rales at the bases of the lungs. As noted in the preceding section, edema of the legs was demonstrated in only 1 case. In 3 cases the lower edge of the liver was palpable well below the costal margin. Since the livers at autopsy were all small with the exception of 1, which weighed 1,490 Gm. but was not palpable clinically, it seems likely that this was due to the decreased capacity of the thorax and the low position of the diaphragm.

Postmortem Observations (*Table*).—In this series the weights of the hearts ranged from 250 to 410 Gm. In normally developed people

3. Barnes, A. R.: Electrocardiographic Patterns, Springfield, Ill., Charles C. Thomas, Publisher, 1940.

the heart weight is best correlated with the body weight. No "normal" figures of heart weights have been published for the deformed persons represented in this series. There are obvious fallacies in applying figures for normal persons to these patients in the determination of cardiac hypertrophy. Comparison of the figures reported here, however, with those established by Smith [4] as normal gives one the definite impression that hypertrophy was present in at least 4 cases. More reliable evidence of hypertrophy is furnished by measurement of the thickness of the right ventricular wall. In 4 cases the hypertrophy was well marked, the thickness of the right ventricle varying from 7 to 14 mm.; in case 2 its measurement equaled that of the left ventricle. In only case 5 could there be any doubt about the right ventricular hypertrophy, since the wall was but 5 mm. thick. Dilatation of the right side of the heart was present in 4 cases. In 2 cases only the ventricle was involved, and in the other 2, both auricle and ventricle suffered. No dilatation could be found in case 5. Hypertrophy of the left ventricle was not seen in any case. Valvular lesions were not observed in any case, nor was any enlargement of the orifices of the tricuspid and pulmonary valves detected. Arteriosclerosis of the coronary arteries in the cases of the 4 older patients was not unusually severe; in 2 cases only was stenosis of a coronary artery present, in both cases in the anterior descending branch. In only 1 case was any gross lesion apparent on section of the myocardium, and that was only a few small patches of fibrosis.

In each case the aorta closely followed the course of the spine, but no significant degree of obstruction was demonstrated.

Small amounts of transudate were frequently present in the pericardial, pleural and peritoneal cavities.

In all patients the lungs were small; the combined weight of the two lungs varied from 530 to 790 Gm. Several factors affect the evaluation of these figures. Congestion and edema, which increase the weight, were present in all. On the other hand, the fact that these persons were underdeveloped must be taken into account in estimating what would be normal for them. In cases 4 and 5, in which the patients were observed personally, one had the definite impression that there was less than the normal amount of lung tissue present. Atherosclerosis was moderate to severe in the larger branches of the pulmonary artery in 4 of the 5 cases. In case 4 no intimal changes were seen. Pulmonary infection was not a factor in any case.

Significant microscopic abnormalities were strikingly lacking. One heart showed fatty degeneration; another had a few areas of fibrosis, and the rest appeared normal. In the lungs no important changes were

4. Smith, H. L.: The Relation of the Weight of the Heart to the Weight of the Body and of the Weight of the Heart to Age, Am. Heart J. **4**:79, 1928.

evident. Collapse, congestion, edema and heart failure cells were noted. The alveolar walls were not thickened. In 4 cases the small pulmonary vessels were normal; in case 5 there was considerable medial hypertrophy of the arterioles.

Congestion of the liver and other organs were the only other relevant findings.

COMMENT

The general clinical pattern in this series of 5 cases was similar to that already described in the literature. Most of these patients survived a longer period than usual, but once acute symptoms developed the disease rapidly progressed to a fatal outcome.

The only fairly constant pathologic findings were hypertrophy and dilatation of the right side of the heart, atherosclerosis of the pulmonary artery and signs of congestive heart failure. These lesions help to explain the mechanism by which extreme spinal deformities cause death.

The investigations of Chapman and his co-workers [1] indicated that the diminution in the size of the lungs and the reduction in the vital capacity with deficient oxygenation of the blood throw an increasing strain on the right side of the heart. Since the pressure in the pulmonary artery cannot as yet be measured, the evidence for increased pressure in the pulmonary vascular tree is indirect and is furnished by the hypertrophied and/or dilated right side of the heart and the atheroma of the pulmonary artery. In the absence of any etiologically important lesions, such as severe pulmonary arteriolar sclerosis, one must, for the present at least, fall back on the concept of a purely mechanical effect on respiratory function to explain the pulmonary hypertension and the failure of the right side of the heart.

SUMMARY

The clinical and pathologic characters of 5 cases of pulmonocardiac failure in extreme spinal deformity are described.

The significant pathologic lesions are the result of pulmonary hypertension, which eventually leads to right-sided heart failure and appears to be due to mechanical interference with respiratory function.

Prof. William Boyd gave me permission to report these cases.

EXCRETION OF COPROPORPHYRIN IN HEPATIC DISEASE

I. CORRELATION OF URINARY AND FECAL EXCRETION WITH PARENCHYMATOUS HEPATIC DAMAGE

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Porphyrins are red pigment substances, the chemical structure of which is based on a ring formed by four pyrrole nuclei which are connected in the form of a closed system by four methine bridges. It has long been recognized [1] that small amounts of porphyrin are excreted normally in the urine of healthy persons and that these amounts may be increased in the urine of patients suffering from a variety of diseases. Porphyrinuria (increased proportion of porphyrin in the urine) has been described as accompanying many forms of hepatic disease,[2] including cinchophen hepatitis, atrophic cirrhosis, obstructive jaundice, chronic passive congestion, lymphosarcoma of the liver, infectious icterus, melanosarcoma of the liver, hemolytic icterus and hemochromatosis.

From the Division of Medicine (Dr. Snell), the Mayo Clinic.

Abstract of a portion of the thesis submitted by Dr. Samuel Nesbitt to the faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy in medicine.

1. (*a*) Garrod, A. E.: On the Occurrence and Detection of Haematoporphyrin in the Urine, J. Physiol. **13**:598-620, 1892. (*b*) Günther, H.: Die Hämatoporphyrie, Deutsches Arch. f. klin. Med. **105**:89-146 (Dec. 20) 1911.

2. Dobriner, K.: Urinary Porphyrins in Disease, J. Biol. Chem. **113**:1-10 (Feb.) 1936. Garrod, A. E.: Haematoporphyrin in Normal Urine, J. Physiol. **17**:349-352, 1894; The Urinary Pigments in Their Pathological Aspects, Lancet **2**:1323-1331 (Nov. 10) 1900; Inborn Errors of Metabolism, ed. 2, London, H. Froude, Hodder & Stoughton, 1923, p. 136. Günther, H.: Haematoporphyrine, in Schittenhelm, A.: Handbuch der Krankheiten des Blutes und der blutbildenden Organe, Berlin, Julius Springer, 1925, vol. 2, pp. 622-673. Tropp, C., and Penew, L.: Quantitativen klinische Harnporphyrinuntersuchungen: II. Lebercirrhosen, Hepatopathien (ausser Cirrhosen), Tuberkulose und andere Krankheiten, Deutsches Arch. f. klin. Med. **180**:411-422, 1937. Vigliani, E. C., and Libowitzky, H.: Ueber Porphyrine im Harn und im Kot, Klin. Wchnschr. **16**:1243-1245 (Sept. 4) 1937; von Zeynek, R.: Zur Frage des einheitlichen Hämatins und einige Erfahrungen über die Eisenabspaltung aus Blutfarbstoff, Ztschr. f. physiol. Chem. **49**: 472-481, 1906. Garrod.[1a]

It has been suggested that porphyrinuria observed in the presence of various other diseases and toxic states is caused by associated hepatic damage or dysfunction.[3] Much evidence has accumulated which would indicate that porphyrin arises in the body during the process of hemopoiesis,[4] rather than during the destruction of hemoglobin, as had been supposed formerly, and that the coproporphyrin which is excreted in the urine and bile represents, first, an amount of isomeric series I porphyrin which arises as a useless by-product of the main synthesis and, second, any isomeric series III porphyrin which has not been utilized in the production of hemoglobin. The rate of excretion of coproporphyrin in the urine and bile depends on several factors, chief of which are the rate of production of the porphyrin which is to be excreted and the efficiency of the liver in disposing of the material. The liver furnishes the most important means of excretion of porphyrins; the kidneys usually excrete only a small fraction of total porphyrins.[5] Various investigators[6] have in fact attempted to devise

3. Althausen, T. L.: Zur Frage der Leberschädigung bei der akuten Porphyrinurie, Klin. Wchnschr. **10**:1016-1017 (May 30) 1931. Fikentscher, R.: Untersuchungen über den Harnporphyrinspiegel bei Schwangerschaftstoxikosen, insbesonders bei Leberstörungen, Ztschr. f. Geburtsh. u. Gynäk. **111**:210-217, 1935. Herold, L.: Das Verhalten der Porphyrinausscheidung bei der Hyperemesis gravidarum und ihre Beziehung zur Leberfunktion, Arch. f. Gynäk. **159**:35-40, 1935. Kapp, E. M., and Coburn, A. F.: Studies on the Excretion of Urinary Porphyrin in Rheumatic Fever, Brit. J. Exper. Path. **17**:255-266 (Aug.) 1936; Spectrographic Observations on Urinary Porphyrins: The Nature of a Combined Form of Coproporphyrin Found in Urine, ibid. **20**:33-41 (Feb.) 1939. Ravn, J.: Stoerung der Leberfunktion bei zwei Faellen akuter Porphyrie nebst einigen Bemerkungen ueber den Begriff Porphyrie, Acta psychiat. et neurol. **10**:459-480, 1935.

4. (*a*) Dobriner, K.: Porphyrin Excretion in Feces in Normal and Pathological Conditions, J. Biol. Chem. **120**:115-127 (Aug.) 1937. (*b*) Dobriner, K., and Rhoads, C. P.: The Metabolism of Blood Pigments in Pernicious Anemia, J. Clin. Investigation **17**:95-103 (Jan.) 1938. (*c*) Dobriner, K.; Strain, W. H.; Localio, S. A.; Keutmann, H., and Stephens, D. I.: II. Coproporphyrin I Metabolism and Hematopoietic Activity, Proc. Soc. Exper. Biol. & Med. **36**: 755-756 (June) 1937. (*d*) Rimington, C.: Porphyrins and Their Relation to Metabolism of Blood Pigments, Proc. Roy. Soc. Med. **32**:1268-1275 (Aug.) 1939. (*e*) Lemberg, R.: Transformation of Haemins into Bile Pigments, Biochem. J. **29**: 1322-1336, 1935.

5. Dobriner, K., and Rhoads, C. P.: Quantitative Determination of Urinary Coproporphyrin, New England J. Med. **219**:1027-1029 (Dec. 29) 1938. Vigliani, E. C., and Waldenström, J.: Untersuchungen über die Porphyrine beim Saturnismus, Deutsches Arch. f. klin. Med. **180**:182-192, 1937. Waldenström, J.: Untersuchungen über Harnfarbstoffe, hapsächlich Porphyrine, mittels der chromatographischen Analyse, ibid. **178**:38-49, 1935. Dobriner.[4a]

6. Brugsch, J. T.: Untersuchungen des quantitiven Porphyrinstoffwechsels beim gesunden und kranken Menschen, Ztschr. f. d. ges. exper. Med. **95**:471-

methods for producing transient porphyrinuria by one means or another as a test of hepatic function.

NATURE OF THE PRESENT STUDY

The present investigation was undertaken to learn whether the degree of porphyrinuria present or the ratio of porphyrins excreted in the urine to porphyrins excreted in the feces in cases of disease of the liver had any relation to the extent of parenchymatous hepatic damage. For this purpose a series of 17 cases was selected. These represented a variety of hepatic diseases and degrees of hepatic damage ranging from mild to severe. Space does not permit an account of the individual cases, some details of which are presented in the table. The patients were maintained on a completely meat-free diet throughout the period of observation. Daily determinations of the amount of coproporphyrin excreted in the urine and feces of these patients were made, often over extended periods because of the extensive fluctuation in values which sometimes occurred from day to day. For this purpose a fluorophotometric method was devised [7] which consisted essentially of successive extraction of a measured portion of a specimen of the urine or stool with an acetic acid-ether mixture and 5 per cent hydrochloric acid, purification of the acid solution by the addition of sodium acetate until it was neutral when tested with congo red paper, repetition of the extraction process and, ultimately, measurement of the amount of coproporphyrin in the final acid extract. This last-named process was accomplished by determination by means of a fluorophotometer [8] of the intensity

481, 1935; Untersuchungen des quantitativen Porphyrinstoffwechsels beim gesunden und kranken Menschen: III. Der Porphyrinstoffwechsel bei Leberenkrankungen, ibid. **95**:493-507, 1935. Franke, K.: Klinische und lebendmikroskopische Untersuchungen der gestörten Leberfunktion: II. Untersuchungen über beginnende Leberschädigungen unter besonderer Berücksichtigung der Porphyrinausscheidung im Urin, Ztschr. f. klin. Med. **130**:222-234, 1936. Franke, K., and Fikentscher, R.: Die Bedeutung der quantitativen Porphyrinbestimmung mit der Lumineszenzmessung für die Prüfung der Leberfunktion und für Ernährungsfragen, München. med. Wchnschr. **1**:171-172 (Jan. 31) 1935. Kaunitz, H.: Ueber Porphyrinurie nach Hämoglobinbelastung, Ztschr. f. klin. Med. **133**:552-562, 1938. Keys, A., and Brugsch, J. T.: Porphyrins and Porphyrinemia, Am. J. Digest. Dis. **5**:49-50 (March) 1938.

7. Nesbitt, S., and Mason, H. L.: Unpublished data.

8. Brugsch, J. T., and Keys, A.: Quantitative Separation and Estimation of Various Porphyrins in Biological Materials, Proc. Soc. Exper. Biol. & Med. **38**:557-560 (May) 1938. Fikentscher, R.: Quantitative Porphyrin-Bestimmung durch Lumineszenzitensitatmessung mit dem Stufenphotometer, Biochem. Ztschr. **249**:357-269, 1932. Stevens, D. S., and Turner, W. J.: The Measurement of Fluorescence Intensity by Photo-Electric Means, J. Lab. & Clin. Med. **23**:81-84 (Oct.) 1937.

of the red fluorescence of the extract in ultraviolet light as compared with the fluorescence of a known standard. In determinations of the fecal excretion of coproporphyrins additional procedures for purification always are necessary to remove porphyrins other than coproporphyrin; this involves several extractions of the porphyrin solution in 0.2 per cent hydrochloric acid with chloroform. By this method normal values for coproporphyrin in the urine were found to range from 0 to 70 micrograms in twenty-four hours, although occasionally values as high as

Clinical and Laboratory Data in Seventeen Cases of Hepatic Disease

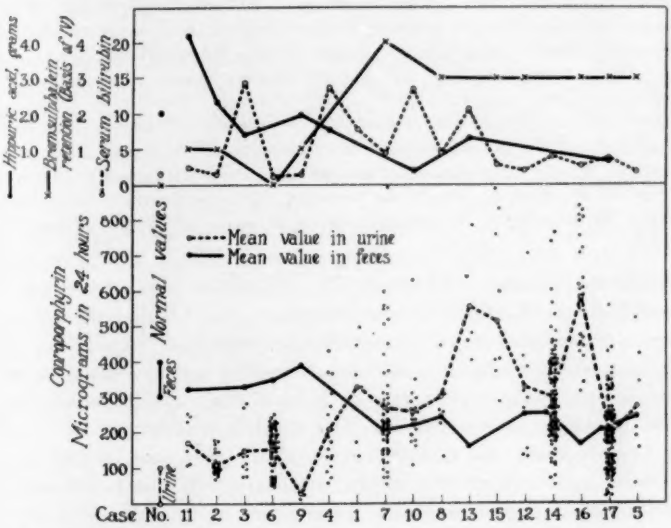
Case No.	Diagnosis	Days Observed	Excretion of Coproporphyrin, Micrograms: In Urine: Range	In Urine: Mean	In Feces, Mean	Urinary-Fecal Ratio of Excretion
1	Obstructive biliary cirrhosis; stricture of common duct	6	132-500	350	.....	.....
2	Obstructive biliary cirrhosis; stone in common duct	21	50-178	105	.....	.....
3	Hepatitis (syphilitic); biliary cirrhosis; stone in common duct	9	76-280	155	312	0.49
4	Epidemic hepatitis	17	58-248	220	.....	.....
5	Hepatic metastasis from carcinoma of rectum	8	190-520	275	246	1.14
6	Fatty liver (gastrocolic fistula)	51	60-240	155	350	0.44
7	Subacute atrophy of liver	49	115-600	275	203	1.40
8	Atrophic cirrhosis of liver (unknown causation)	6	135-445	300	255	1.20
9	Atrophic cirrhosis of liver (unknown causation)	4	18-39	33	395	0.084
10	Atrophic cirrhosis of liver (unknown causation)	17	100-630	270	208	1.3
11	Latent or compensated alcoholic cirrhosis	6	106-242	175	310	0.56
12	Alcoholic cirrhosis	10	53-417	350	245	1.4
13	Alcoholic cirrhosis	5	400-782	570	172	3.3
14	Alcoholic cirrhosis	93	100-600	300	248	1.2
15	Alcoholic cirrhosis	9	290-844	510	.....	.....
16	Alcoholic cirrhosis	48	414-838	595	165	3.6
17	Alcoholic cirrhosis	92	21-560	200	205	0.99

100 micrograms were encountered. The normal range of coproporphyrin in the feces was from 300 to 400 micrograms in twenty-four hours. The urinary-fecal ratio varied from 0.07 to 0.24. Coproporphyrin was isolated from the urine in 10 cases, and its isomeric type was identified by determination of the melting point.[9] In many instances the amount of coproporphyrin present in ascitic fluid and also in the fluid obtained on thoracentesis was determined, and although an appreciable quantity was present in each instance, the amounts in these reservoirs were not sufficient to constitute factors to be considered in the loss of porphyrin from the body.

9. Nesbitt, S.: Unpublished data.

CORRELATION OF LABORATORY DATA AND CLINICAL OBSERVATIONS

In the figure are represented the many determinations of coproporphyrin as excreted in twenty-four hour specimens of urine in each case; the mean value has been indicated in each instance. In most cases the mean value for coproporphyrin excreted in the feces has been included. Whenever possible, certain laboratory determinations, including those of serum bilirubin, retention of bromsulphalein and excretion of hippuric acid, have been represented in order to furnish, so far as possible, an estimation of the degree of parenchymatous hepatic damage


Hippuric acid, grams
Bromsulphalein retention Class at 1'/2
Serum bilirubin
Mean value in urine
Mean value in feces
Coproporphyrin Micrograms in 24 hours
Normal values
Feces
Urine
Case No. 11 2 3 6 9 4 1 7 10 8 13 15 12 14 16 17 5

Excretion of coproporphyrin in the urine and feces in 17 cases of hepatic disease.

present in the individual case. An evaluation of the clinical course of the disease and study of material at necropsy in some instances provided additional information as to the functional capacity of the liver.

An Illustrative Single Case.—We have selected 1 of the 17 cases for consideration in order to illustrate in what manner the aforementioned data were correlated for the purpose of estimating the degree of parenchymatous hepatic damage in relation to the value for the excretion of porphyrins.

In case 17 (table) the patient presented a history of long addiction to alcohol and jaundice and ascites of several months' duration. On physical examination pulsating telangiectasis, icterus, ascites, dependent edema and development of a

collateral circulation were found. The value for serum bilirubin was elevated (3.6 mg. per hundred cubic centimeters), and the reaction to the van den Bergh procedure was direct. A macrocytic type of anemia was noted. The excretion of hippuric acid was 0.72 Gm. in four hours, after the administration of 6 Gm. of sodium benzoate. Results of the bromsulphalein test showed retention of dye of grade 3 (retention of dye graded on the basis of 1 to 4). Peritoneoscopic examination afforded confirmatory evidence as to the correctness of the original diagnosis of atrophic cirrhosis. The course of illness while the patient was under observation also established the extreme extent of parenchymatous hepatic damage. The prothrombin clotting time remained elevated despite parenteral and oral administration of synthetic preparations which possessed vitamin K activity; the value for plasma protein continued to decrease in spite of every effort to sustain it, including intravenous reinfusion of the patient's ascitic fluid. There were also numerous episodes of acute hepatic insufficiency, as evidenced by coma. When the values for coproporphyrin excreted in the twenty-four hour specimens of urine are examined (figure) it is seen that many of them lie well within the normal range. Most of the values, however, are abnormally elevated, to as high as 560 micrograms, and the mean value of these determinations is found to be 200 micrograms, a value considerably higher than values in the normal range. The average daily quantity of coproporphyrin excreted in the feces of this patient for a period of five days was 205 micrograms, an amount definitely below normal. Thus it is seen that the urinary-fecal ratio of the excretion of coproporphyrin in this instance is 0.99, a marked relative increase over the usual value, which ranges from 0.07 to 0.24.

Correlative Factors.—On the basis of a consideration of the data as presented in the figure it at once becomes apparent that the amount of coproporphyrin excreted in the urine in each case from day to day is distributed over a wide range which usually includes some normal values despite severe hepatic damage, so that single determinations may not be considered as informative. However, in all the cases of hepatic disease studied with but a single exception (case 9) most of the values for coproporphyrin excreted in the urine are well above the normal range and the mean value of any given series of determinations seems to be a fair index of the degree of hepatic damage present in the particular case.

As might be expected, there also appears to be a correlation between the amount of coproporphyrin excreted in the feces and the degree of hepatic damage present. In cases 11, 3, 6 and 9, which are representative of moderate hepatic damage, the patients excreted normal amounts of coproporphyrin in the stool, whereas in all the other instances in which determinations of coproporphyrin in feces were carried out, and which are representative of more severely damaged livers, the patients excreted reduced amounts of coproporphyrin in the feces.

Illustrative Laboratory Data.—In case 11 the patient had latent or compensated alcoholic cirrhosis but did not have ascites. The liver was but moderately damaged, as indicated by a slightly elevated value for

serum bilirubin, by a direct reaction to the van den Bergh procedure and by retention of dye of grade 1. The excretion of hippuric acid was normal, and when the patient was seen ten months later the reaction to the test for bilirubin in the serum was indirect and there was no retention of dye. The mean value for a series of estimations of the content of coproporphyrin in the feces of this patient was normal.

In case 2 stone of the common bile duct was present, and the patient showed some degree of biliary cirrhosis at the time of operation. A moderate degree of hepatic damage was present, as indicated by the direct reaction to the van den Bergh test and retention of dye of grade 1; the excretion of hippuric acid was just within normal limits. The mean value for coproporphyrin in the urine was just above normal limits.

In case 3, in which syphilitic hepatitis, stone of the common bile duct and biliary cirrhosis were present, the patient had a large nodular liver; the excretion of hippuric acid was moderately decreased. In this instance the mean value for coproporphyrin excreted in the urine was elevated moderately and the amount excreted in the feces was normal.

In case 6 the patient had a gastrocolic fistula. A diagnosis of fatty liver was made on the basis of nutritional disturbance and was confirmed at operation, although little parenchymatous damage could be demonstrated by the usual laboratory methods. The mean value for coproporphyrin excreted in the urine was only moderately elevated, and the value for coproporphyrin excreted in the feces was normal.

In case 9 the patient had atrophic cirrhosis and presented the clinical picture of cirrhosis, but the liver was functioning fairly well, as was evidenced by retention of dye of only grade 1 and an excretion of hippuric acid of 1.96 Gm. In fact, this man presented an interesting problem in differential diagnosis between constrictive pericarditis and cirrhosis of the liver until determinations of venous pressure had shown normal values and the liver had been visualized by peritoneoscopy. In this instance the mean values for coproporphyrin excreted both in the urine and in the feces were well within normal limits. The patient is gradually improving at the time of this report (June 1941).

In case 4, in which epidemic, or infectious, hepatitis was present, there was a considerable degree of parenchymatous hepatic damage, as evidenced by the elevated content of bilirubin in the serum, the direct reaction to the van den Bergh procedure, an excretion of hippuric acid of 1.52 Gm. and an excretion of galactose of 4.2 Gm. In this instance the mean value for coproporphyrin excreted in the urine was elevated slightly more than it had been in any of the foregoing cases, being approximately 220 micrograms.

In case 1 the patient had stricture of the common bile duct and presented a considerable degree of obstructive biliary cirrhosis, as observed at the time of operation. The mean value for coproporphyrin excreted in the urine was more than 300 micrograms.

In case 7 the patient had subacute atrophy and had sustained a considerable degree of parenchymatous hepatic damage, as evidenced by a direct reaction to the van den Bergh procedure, with a value for serum bilirubin of 4.3 mg. per hundred cubic centimeters, a persistently elevated prothrombin clotting time and retention of dye of grade 4. The excretion of coproporphyrin in the urine was considerably increased, whereas the values for coproporphyrin in the feces were low.

In cases 8 and 10 the patients had atrophic cirrhosis and the livers undoubtedly had sustained considerable parenchymatous damage, as shown by the clinical observations and by laboratory data pertaining to hepatic function. The values for the excretion of coproporphyrin in the urine and feces in these 2 cases are seen in the figure to correspond roughly to analogous values in case 7.

In cases 12, 13, 14, 15, 16 and 17 the condition of advanced alcoholic cirrhosis with extreme degrees of parenchymatous hepatic damage, as evidenced clinically and by laboratory tests, is represented. Case 17, which has been described in some detail, is representative of this group as a whole. It is in this particular group of cases that the highest mean values for coproporphyrin excreted in the urine were found and that the lowest values for coproporphyrin excreted in the feces were encountered. Surprisingly enough, the 1 patient of this group (case 16) who did well and who continued to improve was the one who excreted the greatest quantities (on a mean basis) of coproporphyrin in the urine and whose values for coproporphyrin in the feces were low. The patient who had carcinoma of the rectum with extensive metastasis to the liver (case 5) exhibited both clinical and laboratory evidence of severe hepatic insufficiency, and this condition was confirmed at necropsy.

SUMMARY AND CONCLUSIONS

In 5 cases (11, 2, 3, 6 and 9) in which evidence of mild hepatic damage was presented the mean value for coproporphyrin excreted in the urine during twenty-four hours was between 100 and 200 micrograms in 4 instances and was well within normal limits in the other case. In 4 of these cases the excretion of coproporphyrin in the feces was within normal limits. In 5 cases (4, 1, 7, 10 and 8) in which evidence of moderately severe parenchymatous hepatic damage was presented the mean value for the excretion of coproporphyrin in the urine ranged between 200 and 300 micrograms per twenty-four hours with a single exception, in which the excretion was 350 micrograms per twenty-four hours. In 3 of these cases (7, 10 and 8) the excretion of

coproporphyrin in the feces was diminished; it ranged between 200 and 255 micrograms per twenty-four hours. In 7 cases (13, 15, 12, 14, 16, 17 and 5) in which there was a severe degree of parenchymatous hepatic damage the patients were found with 2 exceptions to have a mean value for excretion of coproporphyrin in the urine which ranged from 300 to 600 micrograms per twenty-four hours. The 2 excepted patients had lower values of 200 and 275 micrograms, respectively. The content of coproporphyrin excreted in the feces of 6 of these patients was found to be diminished and ranged from 160 to 260 micrograms per twenty-four hours. It would seem that among patients suffering from various diseases of the liver the degree of porphyrinuria present and particularly the urinary-fecal ratio of the excretion of coproporphyrin are fair indexes of the extent of parenchymatous hepatic damage present at the time of investigation. The data on the excretion of coproporphyrin are not necessarily of prognostic value, as shown by the occasional recovery of patients in whom the urinary-fecal ratio of such excretion was much disturbed.

NOTE.—Since this work was completed a recently reported investigation, the results of which are in accord with our observations, has come to our attention.[10]

10. Localio, S. A.; Schwartz, M. S., and Gannon, C.: The Urinary/Fecal Coproporphyrin Ratio in Liver Disease, J. Clin. Investigation **20**:7-11 (Jan.) 1941.

EXCRETION OF COPROPORPHYRIN IN HEPATIC DISEASE

II. URINARY AND FECAL EXCRETION IN BILIARY OBSTRUCTION

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It has been suggested by various workers that porphyrin concerned in body metabolism arises during the process of formation rather than destruction of hemoglobin [1] and that the rate of excretion of coproporphyrin in the urine and bile depends chiefly on the rate of production of porphyrin to be excreted (a series I isomer arising as a useless by-product and any series III isomer not immediately utilized in hemopoiesis) and the efficiency of the liver, which is the more important organ involved in excretion of porphyrin.[2] The kidneys normally excrete a small fraction of the total porphyrin excreted, but in the event of hepatic damage they excrete increased amounts, a mechanism similar to that for the excretion of bile pigments. It has been claimed that in

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Abstract of a portion of the thesis submitted by Dr. Nesbitt to the faculty of the Graduate School of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy in medicine.

1. (*a*) Dobriner, K.: Porphyrin Excretion in the Feces in Normal and Pathological Conditions, J. Biol. Chem. **120**:115-127 (Aug.) 1937. (*b*) Dobriner, K., and Rhoads, C. P.: The Metabolism of Blood Pigments in Pernicious Anemia, J. Clin. Investigation **17**:95-103 (Jan.) 1938. (*c*) Dobriner, K.; Strain, W. H.; Localio, S. A.; Keutmann, H., and Stephens, D. I.: II. Coproporphyrin I Metabolism and Hematopoietic Activity, Proc. Soc. Exper. Biol. & Med. **36**:755-756 (June) 1937. (*d*) Rimington, C.: Porphyrins and Their Relation to the Metabolism of Blood Pigments, Proc. Roy. Soc. Med. **32**:1268-1275 (Aug.) 1939. (*e*) Lemberg, R.: Transformation of Haemins into Bile Pigments, Biochem. J. **29**:1322-1336, 1935.

2. (*a*) Dobriner, K., and Rhoads, C. P.: The Quantitative Determination of Urinary Coproporphyrin, New England J. Med. **219**:1027-1029 (Dec. 29) 1938. (*b*) Watson, C. J.: Concerning the Naturally Occurring Porphyrins: I. The Isolation of Coproporphyrin I from the Urine in a Case of Cincophen Cirrhosis, J. Clin. Investigation **14**:106-109 (Jan.) 1935; (*c*) Concerning the Naturally Occurring Phorphyrins: IV. The Urinary Porphyrin in Lead Poisoning as Contrasted with that Excreted Normally and in Other Diseases, ibid. **15**:327-334 (May) 1936. Dobriner.[1a]

complete obstruction of the common bile duct the total excretion of coproporphyrin is not altered but that coproporphyrin practically or entirely disappears from the feces and appears in greatly increased amounts in the urine [3] and occurs in the blood serum, which normally does not contain any coproporphyrin.[4]

Certain experimental procedures have been carried out which lend support to such a mechanism of excretion of porphyrin.[5] Lageder [5b] studied the bile pigment values along with the urinary excretion of porphyrin by rabbits and concluded that the porphyrinuria which developed after ligation of the common bile duct was not explainable simply as an overflow but that some correlation existed between the porphyrinuria and the degree of damage of the liver cells. This is borne out by the fact that Watson [2b] in 3 cases of obstruction of the common duct (stone) noted a definite but only moderate increase in the urinary coproporphyrin, which was much less than the porphyrin in the urine in a case of cinchophen cirrhosis. On the other hand, the urine of a patient who had hepatic insufficiency due to advanced hepar lobatum did not contain any trace of coproporphyrin. This might indicate that more than one factor operates in the excretion of coproporphyrin by the liver.

In order to investigate the urinary-fecal ratio of coproporphyrin excretion associated with obstruction of the common bile duct, 6 cases were selected which represented various degrees of obstructive jaundice as well as various degrees of parenchymatous hepatic damage caused by neoplasm, stricture of the common duct or calculus of the common duct. The relation of the amount of coproporphyrin excreted in the urine in these cases as determined in the twenty-four hour specimens to that excreted in the feces is demonstrated graphically in figures 1 through 6. In these cases the patients were studied before and after operative procedures which were carried out to restore continuity of the bile

3. Brugsch, J. T.: Untersuchungen des quantitativen Porphyrinstoffwechsels beim gesunden und kranken Menschen: VIII. Zur fluorometrischen Kennzeichnung, Auftrennung und quantitativen Berechnung von Porphyringemischen des normalen und krankhaften menschlichen Stoffwechsels, Ztschr. f. d. ges. exper. Med. **103**: 518-538, 1938. Keys, A., and Brugsch, J. T.: Porphyrins and Porphyrinemia, Am. J. Digest. Dis. **5**:49-50 (March) 1938.

4. Boas, I.: Ueber das Vorkommen von Protoporphyrin im Harn, Klin. Wchnschr. **12**:589-591 (April 15) 1933.

5. (*a*) Mathews, F. P.: Photosensitization and the Photodynamic Diseases of Man and the Lower Animals, Arch. Path. **23**:399-429 (March) 1937. (*b*) Lageder, K.: Klinische Porphyrinuntersuchungen mit einer quantitativen spektroskopischen Methode, Arch. f. Verdauungskr. **56**:237-256 (Nov.) 1934. (*c*) Smetana, H.: Studies on Photodynamic Action: II. The Fate of Hematoporphyrin After Parenteral Administration; III. The Influence of Sensitizer on Photooxidation of Tissues, J. Biol. Chem. **125**:741-751 (Oct.) 1938. (*d*) Eldahl, A.: Porphyrines and Clinical Methods for Their Demonstration, Acta med. Scandinav. **97**:538-546, 1938.

passage. It must be realized that it is difficult or impossible to be sure of a completely obstructed bile passage because duodenal drainage is not entirely reliable, and one must be guarded in drawing conclusions from data derived from surgical patients such as these because of the many complicating factors present, for example, the anesthetic agent employed, most anesthetic substances being known to affect excretion of porphyrin.

Case 1 (fig. 1) illustrates the values which might be expected in the instance of obstruction of the common duct with little or no parenchymatous hepatic damage. The patient presented clinical evidence of obstruction for only two weeks. The obstruction was found at operation to be caused by carcinoma of the head of the pancreas. Before operation the stools did not contain any coproporphyrin and the urinary excretion of coproporphyrin was elevated. After cholecystogastrostomy the patient excreted normal amounts of coproporphyrin in the stool and the urinary excretion fell within normal limits.

In case 2 (fig. 2), in which obstruction of the common duct was caused by carcinoma of the pancreas, the course was similar, although in this instance, even before operation, considerable coproporphyrin was excreted in the feces and the urinary excretion was not markedly elevated, perhaps indicating that obstruction was not complete.

Case 3 (fig. 3), in which obstruction of the common bile duct was caused by stricture, illustrates the usual elevated excretion of coproporphyrin in the urine, there being a small amount in the feces. Subsequent to hepaticoduodenostomy the excretion of coproporphyrin in the feces increased, but by the time the patient was dismissed the urinary excretion had not quite returned to within normal limits, perhaps because of parenchymatous hepatic damage which was known to exist.

Case 4 (fig. 4), in which there was a calculus in the common duct, illustrates what might be expected to occur in the instance of partial or intermittent biliary obstruction. The urinary excretion of coproporphyrin varied from day to day from high normal values to only moderately elevated levels, and considerable quantities of coproporphyrin were excreted in the stool. Subsequent to removal of the calculus from the common duct there was but little change in excretion of porphyrin, there being a slight increase in the fecal excretion of coproporphyrin and a tendency for the urinary excretion to remain at a lower level.

Case 5 (fig. 5) is that of a woman who had obstruction of the common bile duct by carcinoma of the duct itself. Despite the fact that bile was not obtained by duodenal drainage, there was an appreciable quantity of coproporphyrin in the stool, which perhaps may mean that some bile did pass through at times. There is evidence that bacteria in the intestine may synthesize coproporphyrin or may produce it from

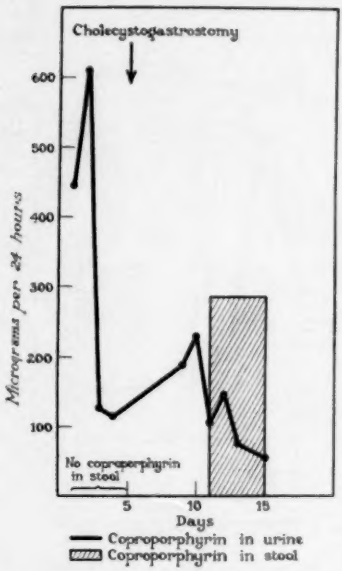

Fig. 1 (case 1).—Urinary and fecal excretion of coproporphyrin before and after relief of biliary obstruction.

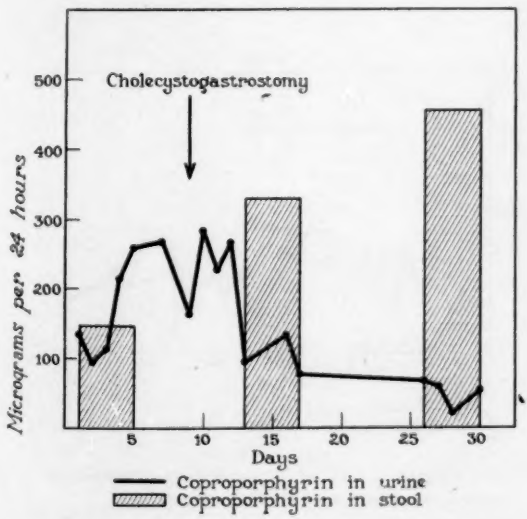

Fig. 2 (case 2).—Urinary and fecal excretion of coproporphyrin before and after relief of biliary obstruction. The obstruction was perhaps not complete.

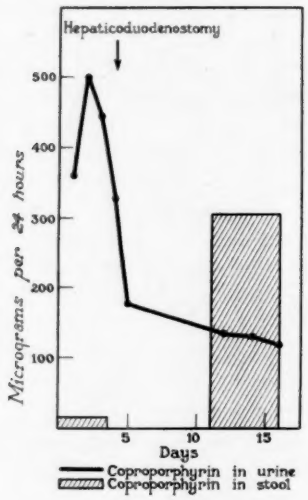

Fig. 3 (case 3).—Urinary and fecal excretion of coproporhyrin before and after relief of biliary obstruction. The failure of the urinary excretion to return to normal may have been due to parenchymatous hepatic damage.

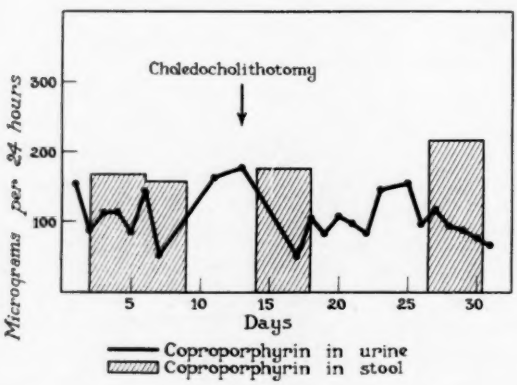

Fig. 4 (case 4).—Urinary and fecal excretion of coproporyhyrin before and after relief of partial or intermittent biliary obstruction.

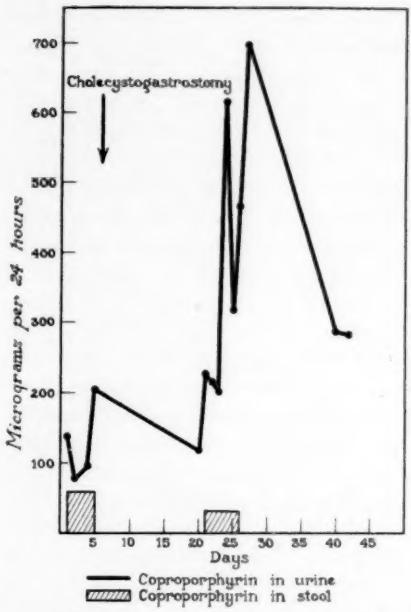

Fig. 5 (case 5).—Urinary and fecal excretion of coproporphyrin before and after relief of biliary obstruction. The increase of urinary excretion and decrease of fecal excretion after operation may have been due to further parenchymatous hepatic damage.

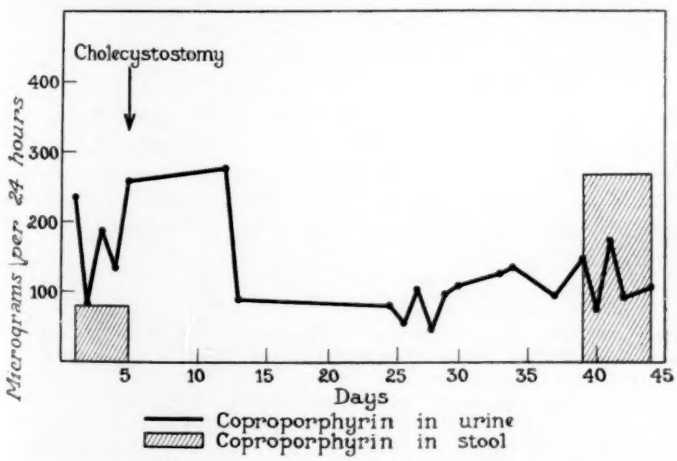

Fig. 6 (case 6).—Urinary and fecal excretion of coproporphyrin. For further explanation see the text.

blood [6] in the intestine, although the idea has been opposed by Kämmerer.[7] If this was so, it would constitute a possible explanation for the unexpected or abnormal amounts of coproporphyrin encountered in the feces of certain of these patients. Subsequent to restoration of the bile passage by means of cholecystogastrostomy, the excretion of coproporphyrin in the urine was increased, whereas that in the feces was decreased. This may be accounted for by the fact that further parenchymatous hepatic damage due to ascending infection, cholangitis or biliary cirrhosis occurred, so that less coproporphyrin was excreted by the liver and the kidneys excreted the excess into the urine. This suggestion is supported by the fact that the patient exhibited every evidence of cholangitis and hepatic insufficiency and that she declined rapidly and died a short time after returning to her home.

Case 6 (fig. 6), in which biliary obstruction was caused by carcinoma of the gallbladder, presented interesting features. The patient, a woman, had an external biliary fistula and was ingesting what bile could be obtained, which probably explains the presence of considerable coproporphyrin in the feces and the fact that the initial urinary excretion was not greatly elevated, a considerable fraction being excreted in the bile through the fistulous tract. After cholecystostomy the amount of coproporphyrin in the feces increased as would be expected, but the urinary excretion did not fall within normal limits, probably because of the extensive hepatic necrosis which was present.

SUMMARY AND CONCLUSIONS

The amount of coproporphyrin excreted daily in the urine and feces in 6 cases of obstruction of the common bile duct of varying severity has been estimated both before and after operative procedures which were done to restore the continuity of the bile passage. Before operation the ratio of the amount of coproporphyrin excreted in the urine to the amount excreted in the feces was found to be increased in proportion to the degree of biliary obstruction. Subsequent to operation the urinary-fecal ratio of excretion of coproporphyrin returned to normal or nearly normal or remained elevated, apparently depending on the degree of parenchymatous hepatic damage.

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6. Jakob, A.: Ueber den Abbau von Blutfarbstoff durch Reinkulturen von Bacterien und über die biologische Synthese von "Koproporphyrin III," Klin. Wchnschr. **18**:507-508 (April 8) 1939; Ueber den Abbau von Blutfarbstoff zu Porphyrinen durch Reinkulturen von Bacterien und über eine neue biologische Synthese von Koproporhyrin III, ibid. **18**:1024-1028 (July 29) 1939.

7. Kämmerer, H.: Ueber den Abbau von Blutfarbstoff zu Porphyrinen durch Reinkulturen von Bacterien und über eine neue biologische Synthese von Koproporhyrin III, Klin. Wchnschr. **18**:1323 (Oct. 7) 1939.

HAMARTIAL NATURE OF THE TUBEROUS SCLEROSIS COMPLEX AND ITS BEARING ON THE TUMOR PROBLEM

REPORT OF A CASE WITH TUMOR ANOMALY OF THE KIDNEY AND ADENOMA SEBACEUM

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NEW YORK

Tuberous sclerosis was first described clinically by Bourneville and Brissaud (1880 *et seq*)[1] in feeble-minded children with epilepsy and progressive mental deterioration. This interesting condition has usually been classified among the diseases of the nervous system and has been given only casual attention by general clinicians. Since the term tuberous sclerosis refers primarily to the changes in the brain, the broader term tuberous sclerosis complex is therefore employed to denote the coexistence of homologous changes in other organs, notably the heart, kidneys and skin.

The clinical diagnosis is possible if two or more of the following features can be demonstrated: mental retardation, epilepsy, adenoma sebaceum, phacoma of the retina, multiple mixed tumors (hamartomas) of the kidneys and a familial history of the disease. In some of the reported cases the neurologic features were insignificant or lacking clinically and were discovered first at autopsy.[2] In still other cases the brain apparently presented nothing abnormal despite well marked expressions of the disease in other organs, such as the heart,[3] kidneys [4]

From the Pathological Laboratory, St. Peter's Hospital, New Brunswick, N. J.

1. (*a*) Bourneville, D. M.: Contribution à l'étude de l'idiotie. Sclérose tubéreuse des circonvolutions cérébrales; idiotie et épilepsie hémiplégique, Arch. de neurol. **1**:81, 1880. (*b*) Bourneville, D. M., and Brissaud, E.: Encéphalite ou sclérose tubéreuse des circonvolutions cérébrales, ibid. **1**:297, 1880. (*c*) Bourneville, D. M.: Sclérose cérébrale hypertrophique ou tubéreuse compliquée de méningite, Progrès méd. **3**:129, 1896; (*d*) Idiotie symptomatique de sclérose tubéreuse ou hypertrophique, ibid. **10**:241, 1899; (*e*) Idiotie et épilepsie symptomatiques de sclérose tubéreuse ou hypertrophique, Arch. de neurol. **10**:29, 1900.

2. Feriz, H.: Ein Beitrag zur Histopathologie der tuberösen Sklerose, Virchows Arch. f. path. Anat. **278**:690, 1930.

3. (*a*) Rehder, H.: Ein Beitrag zur Kenntnis der sogenannten Rhabdomyome des Herzens, Virchows Arch. f. path. Anat. **217**:174, 1914. (*b*) Cagnetto, cited by Kaufmann,[4] vol. 1, p. 63.

4. Kaufmann, E.: Lehrbuch der speciellen pathologischen Anatomie, ed. 7-8, Berlin, Walter de Gruyter & Co., 1922, vol. 2, p. 1092.

or skin.[5] The presence of the disease may first be suspected, therefore, by the dermatologist or the urologic surgeon. A case of the latter type is reported here.

REPORT OF CASE

F. H., a white girl aged 15, was brought to St. Peter's Hospital Jan. 23, 1933, for treatment of hematuria. Four days previously, while roller skating in follow-the-leader formation, she and several companions lost their balance and fell. Being the last in line, she received their combined weights on her abdomen. She felt pain at once in her back, but when it passed away she resumed play. About four hours later, as she prepared to retire, she passed bloody urine. The following

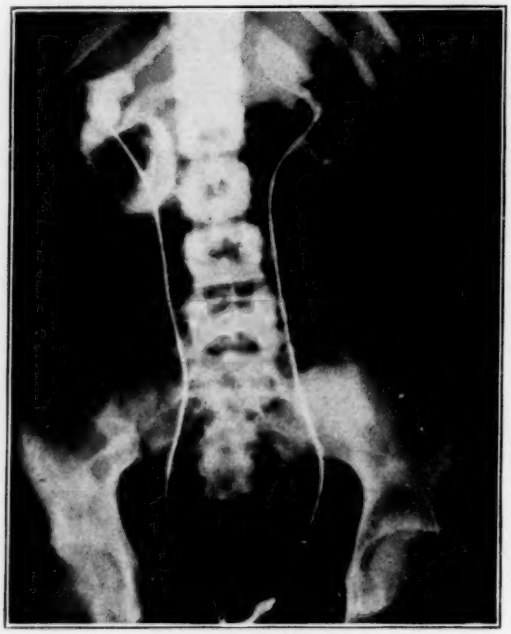

Fig. 1.—The pyelogram made before operation. The dilated right renal pelvis filled unevenly and communicated with an enormous anomalous calix showing a filling defect (blood clot).

morning she voided nearly pure blood and complained of pain in the right groin. She was feverish and vomited several times. From then until the time of admission to the hospital she continued to pass bloody urine intermittently.

A pyelogram (fig. 1) disclosed uneven filling and considerable dilatation of the pelvis and calices of the right kidney. The calix to the lower pole was apparently replaced by a large rounded cavity the size of a plum. This seemed to be outside the kidney within a large mass. The latter had a well defined rounded

5. Olson, G. M.: Adenoma Sebaceum and Tuberose Sclerosis, Arch. Dermat. & Syph. **6**:21 (July) 1922. Pasini, cited by Ferraro and Doolittle.[26]

contour and lay below and mesial to the kidney and overlapped its lower pole. A diagnosis of bleeding tumor was made, and the kidney was resected through a lumbar incision by Dr. R. L. McKiernan.

The specimen removed at operation consisted of the right kidney, which presented a curious ovoid tumor mass springing from its anterior surface just above its lower pole. The mass, roughly one third the size of the kidney itself, was smooth, had rounded overlapping edges and covered the lower part of the kidney like the head of a large mushroom. On section it appeared to be formed by the anomalous expansion of the entire lower anterior segment of the kidney. Its

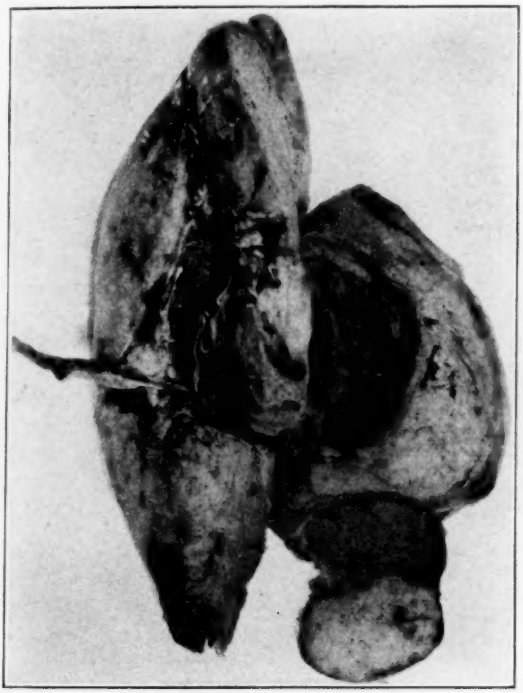

Fig. 2.—The resected right kidney opened to show the large tumor mass, the center of which is formed by the anomalous calix (the blood clot has been removed). Note also the separate smaller tumor mass at the lower pole.

cut surface was yellowish white. Its central portion was hollow and took the form of an anomalous caliceal outgrowth of the renal pelvis. This cavity and the pelvis proper were distended with clotted blood. Its lining was smooth and was continuous with the mucosa of the pelvis (fig. 2). A separate smaller mass, the size of a walnut kernel, was also noted overlying the lower pole of the kidney. It had the same consistency and appearance on section as the larger mass.

Microscopic examination of the tumor masses revealed a mixture of several kinds of unripe tissue of mesenchymal origin. Among these could be defined spindle cell types, smooth muscle, numerous small and large thick-walled blood channels (figs. 3 and 4) and some fat tissue (fig. 5). The cells appeared somewhat atypical but without evidence of active proliferation. Epithelial elements resembling tubules were not encountered. Sections taken from the rest of the kidney revealed a number of minute cysts, probably belonging to the tubular system. They were lined by well formed cuboidal cells with abundant clear cytoplasm.

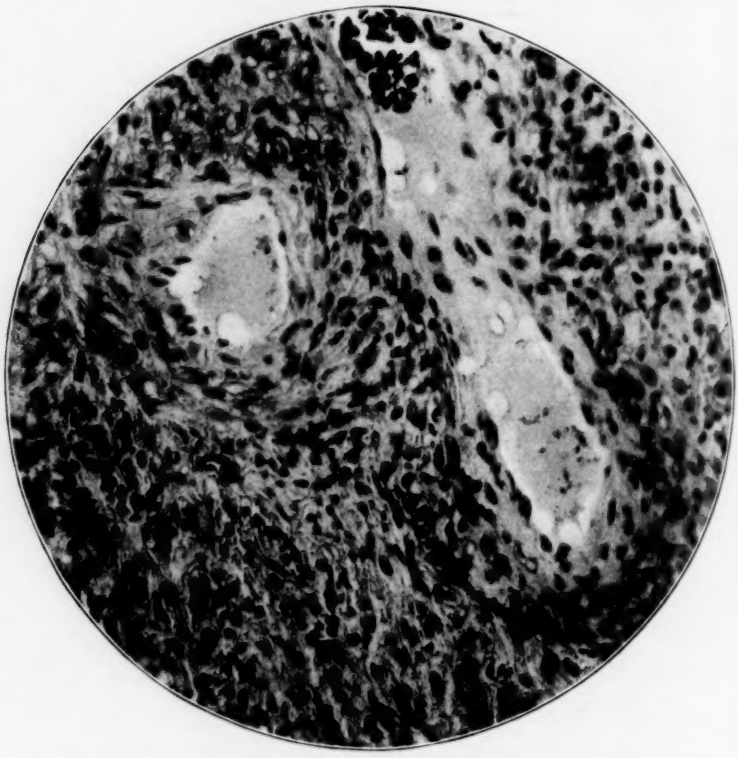

Fig. 3.—A photomicrograph of the hamartoma, showing the primitive mesenchymal cell composition with predominance of vasoformative tissue.

These cysts varied considerably in size and shape and were surrounded by a well developed connective tissue stroma. They appeared to be anomalous rather than obstructive in origin (fig. 6).

The pathologic diagnosis was bleeding hamartoma of the kidney with a caliceal anomaly. The surgical wound healed quickly, and the patient returned home in good condition. Because of the unusual nature of the pathologic findings in the kidney it was suspected that the patient might also harbor other unusual features. She was therefore referred back to the hospital for a general examination.

Over her face and neck were many shiny wartlike nodules, most numerous along the sides and on the tip of her nose and along the nasolabial folds, converging toward her chin. The lesions were characteristic of adenoma sebaceum (fig. 7*A*). They ranged from pinpoint to matchhead size. Most of them were

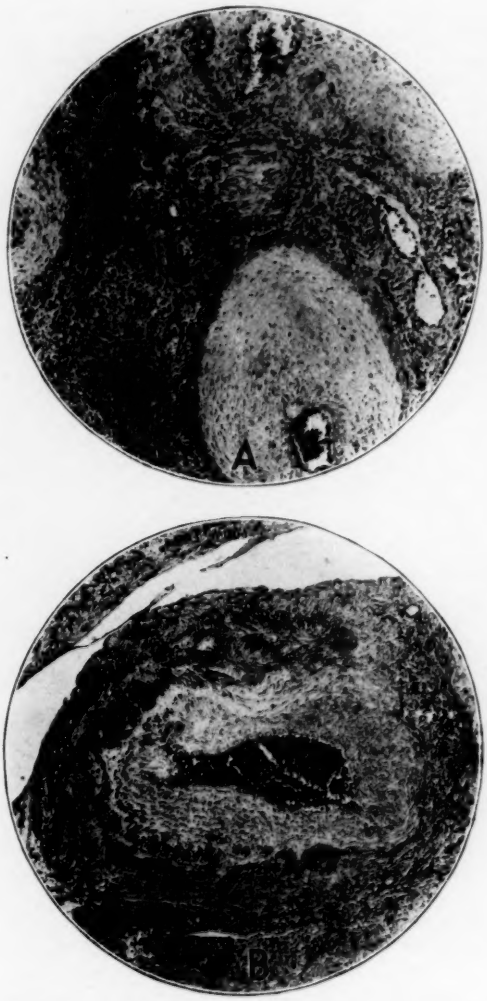

Fig. 4.—Anomalous blood vessels within the hamartoma. *A*, the abnormally thick intima, the eccentric lumen and the indefinite media and adventitia. *B*, an anomalous artery, showing incomplete, though definite, formation of a media and an abnormally thick intima without any elastica.

papular, but some were pedunculate. In places they were confluent. The nodules were pale red except when she blushed, and then they became bright red and more prominent. They were smooth and somewhat greasy. A few grouped lesions were also seen on her forehead, on the back of her neck below the hair line and in front above the right clavicle.

When the patient and her parents were questioned certain additional facts were learned which made the diagnosis of tuberous sclerosis highly probable. When she was about 4 years old "pimples" had appeared on her forehead and face and had soon reached the extent just described. Since the age of 5 and until one year previously she had had occasional "fainting spells," in which she suddenly grew rigid and fell unconscious. She would recover from these in a

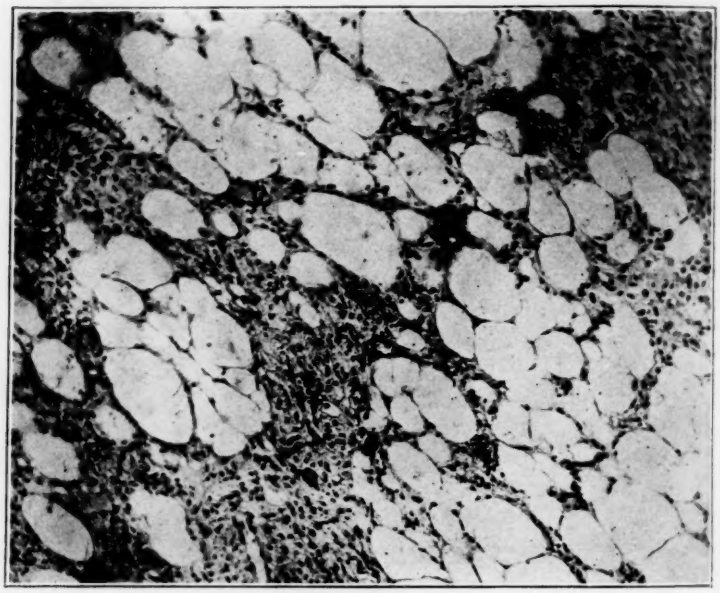

Fig. 5.—Islands of adipose tissue within the hamartoma, showing numerous primitive multinucleated lipoblasts.

few minutes and resume her former activity as if nothing had happened. She had never exhibited violent movements, frothing, tongue biting or incontinence.

She was the eldest of 4 children. The other 3 were apparently entirely well. She had been a normal infant and had begun to talk at the age of 1 year but walked late. She had started to school at the age of 7 and had reached the sixth grade. She was considered slightly backward and had always been "nervous, shy and sensitive." When asked simple questions during the examination she exhibited unusual diffidence. When this was overcome she gave correct answers and showed no gross defect in intelligence.

Her parents were healthy persons of German origin. The family history was lacking in evidence of other instances of the tuberous sclerosis complex, neurofibromatosis or other heredofamilial disorders.

Fig. 6.—Photomicrographs of hamartial "germs" within nontumorous regions of the renal cortex. *A* and *B*, sections containing anomalous cystic tubules lined with cuboidal epithelium of primitive type. *C*, a fibroma-like nodule within the cortex, consisting predominantly of primitive mesenchymal stroma, with few tubules. *D*, the tissue within C at a higher magnification.

On a subsequent visit she was examined ophthalmoscopically. The right disk was obscured by a pearly white elliptic plaque with a smooth protuberant surface and a sharp outline. Its long axis was vertical and slightly larger than the diameter of the disk itself. The vessels were covered by this mass.

On Nov. 30, 1937, nearly five years later, she was readmitted to the hospital for treatment of headache and vomiting. She had been perfectly well in the interval. The onset of these disorders had occurred suddenly three days previously, and they had been accompanied on the following day by drooping of the left eyelid and by periodic lapses into mild stupor. Examination showed divergent strabismus of the left eye. There was considerable ptosis of the left upper eyelid (fig. 7 *B*). The left pupil was somewhat dilated and did not react to light. Some pupillary reaction to accommodation and consensual light stimulation was elicited. Slight paresis of the lower part of the face on the left side was also

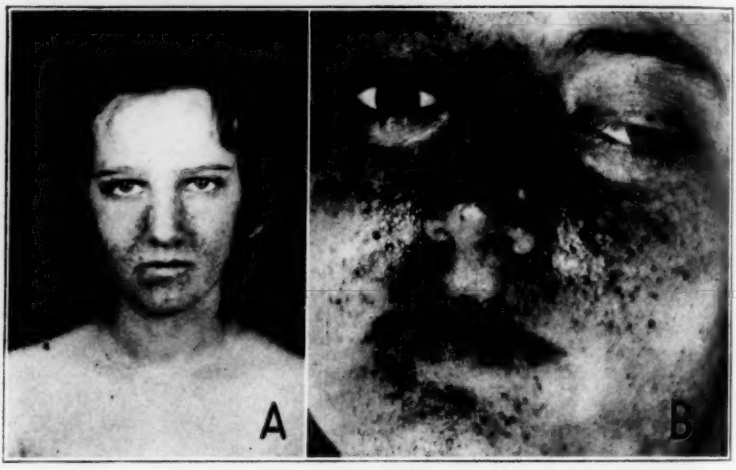

Fig. 7.—*A*, photograph of the patient taken at the age of 15 years, showing adenoma sebaceum. Note the symmetric distribution of lesions on either side of the nose and on the chin, also the scattered lesions on the forehead and neck. *B*, photograph of the patient taken at the age of 20 years, showing a slight increase in the size and extent of the cutaneous lesions, also ptosis and external strabismus of the left eye.

noted. Both fundi exhibited swelling of the nerve head, which was more marked in the left eye. The retinal plaque was much less apparent on the swollen right disk. No other significant neurologic findings were detected. Treatment consisted of dehydrating measures to relieve increased intracranial pressure. During the three weeks of observation in the hospital her condition improved. The neurologic findings varied from time to time in degree, but the headaches and vomiting largely subsided. Periodic drowsiness continued, and the oculomotor changes oscillated from mild to severe. In the opinion of observers the neurologic symptoms were explained by tuberous foci in the left quadrigeminal body which

encroached on the aqueduct of Sylvius, accounting for the left ophthalmoplegia with Argyll Robertson pupil, the somnolence and the precipitate occurrence of internal hydrocephalus and choked disk. Surgical intervention was considered unwarranted, and the patient was discharged at her own request. She was subsequently admitted to another hospital, where a decompression was performed. She died at home a short time later.

PATHOLOGIC AND CLINICAL OBSERVATIONS

Renal Involvement.—According to the available data, renal tuberosities affect more than half the persons who have the tuberous sclerosis complex.[6] Because of their small size they are generally latent clinically and are an incidental finding at autopsy. Rarely, however, their bulk and multiplicity are sufficient to cause uremia during the lifetime of the patient. Hemorrhage from the kidney or evidence of suppuration may lead to their discovery ante mortem by means of surgical exploration, as in this case. Pyelography is rarely helpful in their diagnosis because of their size and situation. They may occur in the cortex in the form of multiple discrete nodules of varying size, or they may be agglomerated into a single extensive irregular knobby mass. In some instances part or all of one kidney presents a uniform expansion. Individual nodules are sharply circumscribed and may project prominently above the surface. Their color ranges from grayish white or grayish red to yellowish white or yellow. They may be soft or firm. In addition, they may be combined with one or more gross developmental anomalies of the kidney. In the case reported here the extirpated kidney presented a mushroom-shaped tumor-like overgrowth of its lower third. It was yellowish white and rather firm. Its center was occupied by a large round cavity representing the corresponding, anomalous calix, previously visualized in the pyelogram. An additional, smaller tumor overlay the lower pole. Many anomalous cystic tubules within thickened stroma were seen histologically in sections from other parts of the kidney, which were free of gross abnormality.

Poorly differentiated cells of spindle or myoblast type, as typified in the present case, make up the bulk of these nodules. The cells are fairly uniform and have regular nuclei of medium size and round or oval shape. Despite their primitive aspect, they show no evidence of active proliferation. The intercellular substance is moderate in amount and stains poorly; it suggests a primitive sort of collagen or myoglia. Islands of fat are scattered through the nodules and in some instances comprise the greater part of their substance. Multinucleated steatoblasts may be seen in and around the fully formed fat (fig. 5). The vascular components are well developed and striking. They may predominate, giving the nodule a distinctly angiomatous character. Their structure is neither distinctively arterial nor venous but resembles that of a cirsoid aneurysm

6. Feriz.[2] Kaufmann.[4]

of the brain [7] or a congenital arteriovenous fistula of an extremity.[8] The vessel walls attain great thickness in spite of an extremely small caliber of lumen. Their layers are formed by the concentric apposition of myoblast cells externally like the annual rings of a tree. In some areas almost the entire tissue seems to be composed of ill defined bundles of vasoformative cells. These are without definite order except where they exhibit circular alinement into groups of serpentine thick-walled small or medium-sized blood channels having extremely small lumens (fig. 4). These vessels are notably deficient in elastic tissue [2] and often undergo hyalinization. Other types of tissue, such as epithelium-lined tubules and striated muscle, occur in rudimentary form and in small amount.[2] The cell composition throughout appears slow growing and noninvasive. The atypical cytologic aspects and architecture often suggest neoplasia; hence the terms angiofibroliposarcoma, fibrolipomyoangioma and fibrolipomyoangiosarcoma applied by some pathologists.[9] A few cases of the tuberous sclerosis complex have been reported in the literature in association with "hypernephroma" [10] or "adenomyosarcoma of Wilms type." It is likely that at least some of these growths belong rightfully with the renal nodules of the tuberous sclerosis complex.[11] When account is taken of their organoid morphology, their limited growth and the underlying nature of the disease, as a whole, the renal nodules take on an aspect which compels one to the view now generally held that they are best classified not as neoplasms but as hamartomas.[12]

7. (*a*) Cushing, H., and Bailey, P.: Tumors Arising from the Blood-Vessels of the Brain: Angiomatous Malformations and Hemangioblastomas, Springfield, Ill., Charles C. Thomas, Publisher, 1928. (*b*) Levine, V.: Angiomatous Malformations of the Brain: Report of Two Cases of Angioma Racemosum, Arch. Path. **15**:340 (March) 1933. (*c*) Turner, O. A.: Arteriovenous Hamartoma of the Brain: Report of a Case with Autopsy, ibid. **24**:223 (Aug.) 1937.

8. Blumgart, H. L., and Ernstene, A. C.: Hemangiectatic Hypertrophy and Congenital Phlebarteriectasis, with Particular Reference to the Diagnostic Importance of the Peripheral Vascular Phenomena, Arch. Int. Med. **49**:599 (April) 1932.

9. Harbitz, F.: Tuberöse Hirnsklerose, gleichzeitig mit Nierengeschwülsten (Myxo-Lipo-Sarkomen) und einer Hautkrankheit (Adenoma sebaceum), Centralbl. f. allg. Path. u. path. Anat. **23**:868, 1912.

10. In justice to some of the earlier writers on this subject, it should be noted that the term hypernephroma was often used generically for the majority of renal tumors and structures of various types (Ewing,[14] p. 781).

11. (*a*) Hyman, A.: The Association of Hypernephroma with Tuberose Brain Sclerosis and Adenoma Sebaceum, J. Urol. **8**:317 (Oct.) 1922. (*b*) Yater, W. M.: Tumors of the Heart and Pericardium, Arch. Int. Med. **48**:627 (Oct.) 1931.

12. The term hamartoma (Albrecht, E.: Ueber Hamartome, Verhandl. d. deutsch. path. Gesellsch. **7**:153, 1904) denotes a tumor-like malformation marked by defects in tissue combination. A well known example is the simple medullary "fibroma" of the kidney. Grossly this is a sharply defined whitish nodule, slightly larger than a pinhead. Microscopically it is a dense aggregate of stromal tissue

True neoplasms in these cases are rare. Only 3 cases of an unmistakable malignant growth (hamartoblastoma) have been reported in which small metastatic deposits were found in other organs (Kirpicznik,[13] Harbitz,[9] Feriz [2]). While it is a fact that areas of perverted cell development are more favorable soil for new growth than are normal tissues,[14] this tendency is so little in evidence in these hamartomas that one is inclined to wonder whether their fetal cell characters do not rather indicate a considerable measure of defectiveness in growth potency. Hamartial "germs" in the form of minute areas of distorted stroma with atypical tubules may be found scattered throughout the otherwise normal parts of the kidney. They may form minute cysts, as in the case reported here.

Isolated hamartomas of the kidney may also occur independently of the tuberous sclerosis complex. The medullary "fibroma" is well known. In addition, solitary lesions of the cortex have been reported under the title benign mixed tumor. They are generally small and are classified by whatever component is predominant, i. e., as hemangioma, lipoma, myoma, fibroma, etc. They are found mostly in adults, and except for infrequent hematuria they are symptomless.[15]

Cerebral Involvement.—The cerebral manifestations of the disease are its most constant feature. Moreover, they reveal themselves clinically far more overtly than do the renal or the cardiac lesions. In a majority of the recorded cases the patients were epileptic,[16] and many exhibited

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arranged formlessly and enclosing a few scattered rudimentary tubules; it is poorly defined from the surrounding normal stroma. A wide variety of organoid structures and "benign tumors" are probably also included in the definition. Theoretically, this should include congenital vascular nevi and angiomas, also glomangiomas and cirsoid aneurysms and certain types of pigmented and keratotic nevi. There is a growing tendency to regard benign tumors in general not as neoplasms but as hamartomas.[77] The cells of a hamartoma share a common embryonal origin with those of the organ in which they are found. They share to some extent also in the growth of the organ and may later retrogress.

13. Kirpicznik, J.: Ein Fall von tuberöser Sklerose und gleichzeitigen multiplen Nierengeschwülsten, Inaug. Dissert., Berlin, G. Reimer, 1910; Virchows Arch. f. path. Anat. **202**:358, 1910.

14. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928, p. 97.

15. Watson, M. C.: Benign Mixed Tumors of the Kidney, Canad. M. A. J. **18**:511 (May) 1928. MacKenzie, D. W., and Hawthorne, A. B.: Hemangioma of the Kidney: A Report of Two Cases and a Brief Résumé of the Literature, J. Urol. **26**:205 (Aug.) 1931. Bell, E. T.: A Classification of Renal Tumors with Observations on the Frequency of the Various Types, ibid. **39**:238 (March) 1938.

16. (*a*) Hartdegen, A.: Ein Fall von multipler Verhärtung des Grosshirns nebst histologisch eigenartigen harten Geschwülsten der Seitenventrikel ("Glioma gangliocellulare") bei einem Neugeborenen, Arch. f. Psychiat. **11**:117, 1881. (*b*) Vogt, H.: Die tuberöse Sklerose, Arch. f. Kinderh. **48**:369, 1908. (*c*) Freeman, W.: Tuberous Sclerosis, Arch. Neurol. & Psychiat. **8**:614 (Dec.) 1922. (*d*) Footnote 1.

mental retardation and even a severe grade of imbecility. An exceedingly wide variety of epileptic phenomena, psychic and emotional defects and neurologic abnormalities, focal or systematized, have been reported. Diagnosis, however, is rarely established clinically without additional evidence, such as adenoma sebaceum of the skin or phacoma of the retina. Papilledema and other signs of internal hydrocephalus occur infrequently and are traced, as a rule, to expanding lesions in the region of the aqueduct of Sylvius or the third ventricle,[17] such as were diagnosed in the case reported here. In several mature subjects clinical signs of the disease were never noted despite proved lesions in the brain at autopsy.[18] It is rather likely that tuberous foci would be found in every instance of the complex in which autopsy was done, including the "forme fruste," if the brain was examined with sufficient care (Feriz [2]). Instances of the disease in infancy have also been recorded.[19] In general, however, there is a delay of several years after birth before the appearance of epilepsy and the other clinical aspects of the disease.[20] A survey of the subject, with an excellent discussion of its clinical neurology, may be found in the paper of Critchley and Earl (1932).[18a]

The morphologic manifestations of the disease in its cerebral localization are variable.[21] The surfaces of the cerebral hemispheres are found to be the seat of areas of peculiar hardening, white or mottled with gray and scattered irregularly. These areas are of varying size and may be nodular and circumscribed but are often indefinite in outline. In addition, entire convolutions or groups of convolutions may be deformed, either narrowed (microgyria) or widened (macrogyria). On section the transition from gray to white matter is indistinct or completely lost in these regions. In over half the cases irregular projections

17. (*a*) Globus, J. H.; Strauss, I., and Selinsky, H.: Das Neurospongioblastom, eine primäre Gehirngeschwulst bei disseminierter Neurospongioblastose (tuberöse Sklerose), Ztschr. f. d. ges. Neurol. u. Psychiat. **140**:1, 1932. (*b*) Globus, J. H.: Neurinome central associé à une sclérose tubéreuse (neurospongioblastose disséminée), Rev. neurol. **2**:1 (July) 1933. (*c*) Stevenson, L. D., and McGowan, L. E.: A Case of Tuberous Sclerosis Involving Chiefly the Cerebellum and Simulating an Expanding Lesion of the Quadrigeminal Plate, Tr. Am. Neurol. A. **63**:26, 1937. (*d*) Liber, A. F.: Tuberous Sclerosis with Cerebellar Involvement and a Colloid Cyst of the Septum Pellucidum, Arch. Path. **26**:753 (Sept.) 1938.

18. (*a*) Critchley, M., and Earl, C. J. C.: Tuberose Sclerosis and Allied Conditions, Brain **55**:311 (Sept.) 1932. (*b*) Feriz.[2]

19. Globus, J. H., and Selinsky, H.: Tuberous Sclerosis in the Infant, Am. J. Dis. Child. **50**:954 (Oct.) 1935.

20. Yakovlev, P. I.: Congenital Morphologic Abnormalities of the Brain in a Case of Abortive Tuberous Sclerosis: Functional Implications and Bearing on Pathogenesis of So-Called Genuine Epilepsy, Arch. Neurol. & Psychiat. **41**:119 (Jan.) 1939.

21. Kaufmann,[4] vol. 2, p. 1479.

occur on the inner surface of the lateral ventricles. These have often been described as resembling candle gutterings. They may be confluent, however, and may form a dense sclerotic layer. Rarely they take the form of large projecting intraventricular tumors. The cerebellum and spinal cord are rarely affected.

Histologically a variety of cell types is found; these are unique in their unusual configuration and in their frequently gigantic size. Despite their malformed appearance, many intermediate types can be traced between finely branched primitive neuroglial cells (spongioblasts) and gigantic structures of ganglion cell type (neuroblasts) with a large nucleus. The glial elements predominate to a marked degree. Probably all these abnormal cells are collateral derivatives of primordial undifferentiated neuroepithelium (Globus and associates [22]).

An essential feature of the cerebral lesions is the evidence of failure of cell migration during embryonal development. The loss of corticomedullary demarcation in the affected gyri, visible grossly, corresponds histologically with an appearance of "profound confusion of the cortical cytoarchitecture" (Yakovlev [20]). The vascular architecture is similarly deformed, so that a poverty of circulation exists in the affected cortex together with a surplus of vessels in the corresponding subcortical white matter.[20] The blood vessels have thickened walls, somewhat like those in the renal nodules, and calcific deposits occur frequently in and around them.

Transitions are frequently found from diffuse gliosis to focal circumscribed glioma-like overgrowths.[23] Although the multiple foci in the brain are undoubtedly hamartial in nature, true neoplasia is nevertheless a not infrequent occurrence. The tumors are often multiple. Histologically they differ from ordinary gliomatous growths in exhibiting traces of ganglion cell elements, so that they represent actually a form of mixed tumor of the brain (neurospongioblastoma, Globus and associates [24]). The relation between neoplasia and the benign hamartial "germs" and thickenings is one of the most interesting aspects of the disease.

Relation Between Tuberous Sclerosis and Brain Tumor.—The case for a heightened incidence of neoplasm in tuberous sclerosis of the brain seems fairly well established as a result of the studies of Biel-

22. (*a*) Globus, J. H.: Primary Neuroectodermal Brain Tumors: Their Transition from Benign to Malignant Forms, J. Mt. Sinai Hosp. **7**:361 (Jan.-Feb.) 1941. (*b*) Footnote 17 *a* and *b*.

23. (*a*) Bielschowsky, M.: Zur Histopathologie und Pathogenese der tuberösen Sklerose, J. f. Psychol. u. Neurol. **30**:167, 1924. (*b*) Kaufmann.[21]

24. Globus, J. H.: Glioneuroma and Spongioneuroblastoma, Forms of Primary Neuroectodermal Tumors of the Brain, Am. J. Cancer **32**:163 (Feb.) 1938; footnote 22.

schowsky,[23a] Globus and associates [25] and other authorities.[26] Bielschowsky expressed a preference for the view that the disease is the result of a widespread and disseminated blastomatous agency which is active in early embryonic life. On this basis he explained not only the primitive and multifarious cell components of the nodules in the brain and other affected organs but the disorder in architecture resulting from failure of normal cell migration. Pellizzi (1901) [27] and subsequent writers [28] have, for the most part, expressed opinions that the essential lesions of the disease itself represent the product of retardation in cell differentiation and migration, hence a form of disseminated congenital malformation (hamartiosis). The foci of sclerosis and of nodular overgrowth of the brain are accordingly viewed as homologous with the hamartomas in the skin, heart and kidneys. Since the weight of evidence is opposed to the view that the latter manifestations of the disease are neoplastic, it is unlikely, therefore, that the cerebral foci are intrinsically neoplastic. Globus [22a] directed attention to the possibility of the transition of growths in the brain from benign to malignant, in accordance with the Cohnheim-Ribbert theory of origin of neoplasms in embryonal rests. If the term embryonal rest is merely restated as hamartial malformation the entire concept of transition of a neoplasm from benign to malignant is simplified. In effect, the basic lesion is hamartial, becoming in turn tumor-like but benign (hamartoma) and truly neoplastic (hamartoblastoma, viz., neurospongioblastoma).

Certain types of evidence will be cited later which enable one to make a special case for the high incidence of cerebral neoplasm in tuberous sclerosis without doing violence to the concept that the underlying basis for the disease is a disseminated tissue malformation (hamartiosis). Briefly stated, defective organization in the embryonic brain is carried into mature existence in the form of unrealized cell potencies for differentiation and of refractoriness to local and neurotrophic organizer influences. These foci are thereby neither integrated in function nor protected against individualistic tendencies in the cells to proliferate and to form tumors. As a result, they constitute a constant latent source of neoplasia in which cell activation may occur at any time in one or more places. This will be referred to again in connection with the etiology of the disease.

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25. Footnote 17 *a* and *b*.

26. Ferraro, A., and Doolittle, G. J.: Tuberous Sclerosis (Diffuse Neurospongioblastosis), Psychiatric Quart. **10**:365 (July) 1936.

27. Pellizzi, cited by Ferraro and Doolittle.[26]

28. (*a*) Yakovlev, P. I., and Guthrie, R. H.: Congenital Ectodermoses (Neurocutaneous Syndromes) in Epileptic Patients, Arch. Neurol. & Psychiat. **26**:1145 (Dec.) 1931. (*b*) Feriz.[2] (*c*) Kirpicznik.[13] (*d*) Footnote 17 *a* and *b*. (*e*) Critchley and Earl.[18a]

Cutaneous Lesions.—Lesions of the skin when present are of preeminent importance in the clinical recognition of the disease. Their best known form, adenoma sebaceum, is seen in about half the cases.[8] Its appearance on the face is striking and pathognomonic. Properly the condition should be designated hamartoma pilosebaceum, having the same background of faulty development as the tuberous lesions in the brain or kidney. It takes the form of closely grouped nodules, with a butterfly distribution along the nasolabial folds on both cheeks, converging below to the chin (fig. 7). The nodules are most numerous on either side of the nose. They are easily distinguished from the lesions of acne, including that caused by prolonged ingestion of a bromide. Scattered nodules also occur on the forehead and neck. The individual lesions are wartlike or papular and yellow or red. In the case reported here they became bright red whenever the patient blushed. In this as in several other reported cases an angiomatous component was apparently prominent (Pringle type [29]). Peculiar changes in the texture and pigmentation of the skin are often noted in isolated areas in other parts of the body.[30] These, too, have diagnostic significance.

Cardiac Involvement.—The so-called "rhabdomyoma" of the heart has been reported in about half the cases in which typical cerebral changes were exhibited.[31] Thorough examination might possibly have disclosed less obvious defects of tissue structure in the heart in nearly every case (Feriz [2]). Conversely, in only half the recorded cases of rhabdomyoma cordis is associated cerebral disease reported (Cagnetto [3b]), despite evidences of the complex in other organs (kidneys, skin, retina). Here, too, the suspicion exists that complete study of the brain with serial sections would have brought to light the typical pattern of cerebral changes.[2] It is probable, however, that this may be said with assurance only of diffuse or multiple rhabdomyomatosis of the heart, whereas a solitary rhabdomyoma may occur either as part of the complex or as an independent lesion (Mönckeberg [31b]).

In the heart, as in other affected organs, the nodules and swellings are now regarded as tissue malformations (hamartomas) and not as neoplasms.[32] The component cells have an appearance suggesting

29. Pringle, J. J.: Ueber einem Fall von kongenitalen Adenoma sebaceum, Monatsh. f. prakt. Dermat. **10**:197, 1890.

30. (*a*) Urbach, E., and Wiedmann, A.: Morbus Pringle und Morbus Recklinghausen: Ihre Beziehungen zueinander, Arch. f. Dermat. u. Syph. **158**:334, 1929. (*b*) Pringle.[29] (*c*) Feriz.[2] (*d*) Footnote 5.

31. (*a*) Yater.[11b] (*b*) Mönckeberg, J. G.: Rhabdomyom des Herzens, in Geschwülste und Parasiten des Myokards und des spezifischen Muskelsystems, in Henke, F., and Lubarsch, O.: Herz und Gefässe, in Handbuch der speziellen-pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2, p. 482.

32. (*a*) Bonome, cited by Mönckeberg.[31b] (*b*) Farber, S.: Congenital Rhabdomyoma of the Heart, Am. J. Path. **7**:105 (March) 1931. (*c*) Rehder.[3a] (*d*) Mönckeberg.[31b]

embryonal heart fibers, but they are often remarkably misshapen and enlarged to grotesque proportions. Numerous scattered "germs" of embryonal heart muscle are often discovered microscopically in areas which are grossly normal.[31b] No tendency to autonomous growth is evident, and the picture here, like that in the brain and kidneys, is apparently that of a "concluded process" (Kirpicznik[18]). The morphologic nature of these structures remains fixed throughout the subject's lifetime except for fibrous replacement or calcification.[31b] Large nodules may cause obstruction of an important channel, such as the trunk of the pulmonary artery,[33] and cause death through heart failure at an early age. In many cases, however, despite considerable involvement, little or no interference with cardiac function occurs.

Painstaking search often reveals many homologous, though less obvious, changes in other parts of the vascular apparatus. Here, too, the aspect is that of a flaw in development. Ill defined thickenings appear in the walls of certain blood vessels, and their myoelastic architecture shows various degrees of imperfection.[34] Certain types of angioma possibly fall into this category as well. In addition, the hamartomas of the kidneys, skin or brain may contain a large angiomatous component.

Retinal Lesions (Syndrome of van der Hoeve[35]).—Tumor-like lesions of the retina have been observed in a number of cases of the tuberous sclerosis complex during life. Like the nodules in the skin they are pathognomonic of the disease. They have usually been found separate from the disk but occasionally occur on the disk itself, as in the case reported here. Their ophthalmoscopic appearance is that of white or gray areas which are round or elliptic. They may be large and appreciably elevated or small and appearing only as flat plaques. They may be smooth or mulberry-like in contour and are sometimes cystic.[36] The term phacoma has been applied to them. Structurally they are hamartomas of predominantly gliogenous nature, containing possibly a few undifferentiated ganglion cells.[37] It is probable that their determination in the primitive optic neuroepithelium takes place from the fourth to the fifth week of embryonic life (Mann[38]).

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33. Wolbach, S. B.: Congenital Rhabdomyoma of the Heart, J. M. Research **11**:495, 1907. Rehder.[3a] Footnote 32 *a* and *b*. Feriz.[2]

34. Böhm, cited by Feriz.[2]

35. van der Hoeve, J.: Augengeschwülste bei der tuberösen Hirnsklerose (Bourneville), Arch. f. Ophth. (*a*) **105**:880, 1921; (*b*) **111**:1, 1923.

36. Tarlau, M., and McGrath, H.: Pathologic Changes in the Fundus Oculi in Tuberous Sclerosis: Clinical and Pathological Report of a Case of Tumor Arising from the Optic Nerve Head with a Review of the Literature, J. Nerv. & Ment. Dis. **92**:22 (July) 1940.

37. Messinger, H. C., and Clarke, B. E.: Retinal Tumors in Tuberous Sclerosis, Arch. Ophth. **18**:1 (July) 1937.

38. Mann, I.: Personal communication to Messinger and Clarke.[37]

Osseous Lesions.—A case of this disease was reported [39] in 1935 in which roentgenograms of the skull revealed numerous indistinct islands of increased density alternating with areas of rarefaction, together with increased thickness and density of the tables of the skull. Comparable changes were also noted in the bones of the hands and feet. The remainder of the skeleton was free of roentgenologic abnormalities except for spina bifida. Since then, other cases of tuberous sclerosis have been reported in which similar observations were made.[40] In addition, a case has been reported[40b] in which roentgenograms revealed peculiar "flowing" hyperostoses along the shafts of certain bones, typical of the condition known as melorheostosis (Léri). The similarities of the bone changes in tuberous sclerosis to those in multiple neurofibromatosis have been pointed out by certain writers and ascribed either to an overlapping of the two syndromes or to a dysontogenetic factor possessed by both in common.

Coexisting Anomalies of Development.—In several of the reported cases important gross developmental defects were found. These included cyst of the septum pellucidum,[17d] spina bifida,[41] cleft palate,[42] accessory lung,[43] malformation of the pancreas,[44] fused kidney with dystopia cruciata,[2] cardiac defects,[45] subaortic stenosis,[18a] subnormal development of the arch of the aorta [27] and congenital ramifying diverticulum of the left ventricle of the heart.[46] Minor developmental anomalies, however, occur in nearly all the cases,[2] as well as so-called "stigmas of degeneracy." [47] From the standpoint of the pathogenesis of the syndrome, these defects are probably nonspecific yet cannot be considered unrelated. Their significance is best understood when discussed in connection with the pathogenesis of the disease.

ETIOLOGY AND PATHOGENESIS

Theories.—Whatever the nature of its pathogenetic mechanism, the disease is clearly inherent in the germ plasm before conception takes

39. Gottlieb, J. S., and Lavine, G. R.: Tuberous Sclerosis with Unusual Lesions of the Bones, Arch. Neurol. & Psychiat. **33**:379 (Feb.) 1935.

40. (*a*) Dalsgaard-Nielsen, T., cited by Hall. (*b*) Hall, G. S.: Tuberose Sclerosis, Rheostosis and Neurofibromatosis, Quart. J. Med. **9**:1 (Jan.) 1940.

41. Hartdegen.[16a] Gottlieb and Lavine.[39]

42. Jonas, W.: Zur Histologie der tuberösen Hirnsklerose an der Hand eines durch Rhabdomyome des Herzens komplizierten Falles, Frankfurt. Ztschr. f. Path. **11**:105, 1912.

43. Kirsch-Hertel, cited by Feriz.[2]

44. Kawamura, R., cited by Farber.[32b]

45. Adrian, C.: Ueber Neurofibromatose und ihre Komplikationen, Beitr. z. klin. Chir. **31**:1, 1901. Bourneville and Brissaud.[1b] Berdez, cited by Feriz.[2]

46. Norman, R. M., and Taylor, A. L.: Congenital Diverticulum of Left Ventricle of Heart in Case of Epiloia, J. Path. & Bact. **50**:61 (Jan.) 1940.

47. Kirpiczink.[13] Critchley and Earl.[18a]

place. It is transmitted generally in accordance with well defined heredofamilial patterns. According to Penrose,[48] its genetic behavior is that of a mendelian dominant. In addition, the disease crops out sporadically in apparently healthy stock, as in the case reported here. Such occurrences are regarded as the result of mutation.

Further analysis of its etiology lies in the realm of hypothesis. According to older views, especially that of Bielschowsky,[23a] the disease is the manifestation of a neoplastic agency active in early embryonal life. On the other hand, the remarkable fidelity to pattern with which the disease reproduces itself in different organs and in different persons, the parallel development of its widely separate lesions, their organoid structure and the relative conformity of their rate of growth and retrogression with that of the host tissues militate strongly against this point of view.

Yakovlev and Guthrie [28a] attempted recently to establish the concept that tuberous sclerosis, like neurofibromatosis and certain other syndromes, is a system disease of neuroectodermal structures (ectoblast). These writers pointed to the simultaneous occurrence of cerebral lesions with adenoma sebaceum of the skin in the first-named condition and of peripheral nerve tumors with cutaneous nevi and, occasionally, adenoma sebaceum in the second one, also to several instances of apparent overlapping between the two syndromes. They explained the occurrence of angiomas and of hamartomas of the kidneys and heart, which are derived from mesodermal elements, as trophic formations determined by abnormalities of the sympathetic nerves. Their evidence is ingenious but not entirely convincing. The visceral lesions of tuberous sclerosis in the heart and kidneys are homologous in every way with the lesions of the skin and brain.

The element of heredity plays a determining role so frequently in the occurrence of the tuberous sclerosis complex that the disease must be regarded basically as the outcome of a genic abnormality within the germinal elements of the affected individual. Theoretically, the determiners of the disease may be assumed to enter into and to disturb the embryonal pattern in several widely separated regions at a selectively predetermined stage of development which is identical in all cases. Some estimates place the time of this disturbance between the third and fourth months of intrauterine life, but there is much authority for placing it earlier.[49] If inferences are drawn from the structure of the lesions themselves, the timing of this event should coincide with a stage of tissue ambivalence in which differentiation has not yet progressed far but in which various organs have already become defined. Among these

48. Penrose, L. S.: The Influence of Heredity on Disease: Buckston Browne Prize Essay, 1933, London, H. K. Lewis & Co., 1934, p. 61.

49. Feriz.[2] Kirpicznik.[13] Mann.[38]

must necessarily be included the metanephros, or future kidney, of which no vestige exists until the fifth week, when the ureteric evagination first appears. Since the renal hamartomas are composed chiefly of derivatives of primitive nephrogenic mesenchyme, it is likely that they owe their inception to a disturbance affecting the organization of the latter about the ends of the primordial collecting tubules. This point of differentiation is not reached normally until about the sixth week.

The frequent occurrence of unrelated developmental anomalies (accessory lung, spina bifida, fused kidney, etc.) gives some basis for the suggestion that the determination of the tuberous sclerosis complex may even begin to take place somewhat prematurely, so that organ differentiation is disturbed as well as cell differentiation (Feriz [2]).

The Role of Embryonic Organizers and Cell Competence in Normal Differentiation and in Malformation.—The pathogenesis of the tuberous sclerosis complex, like that of any developmental anomaly, may be stated to depend, generally speaking, on faulty differentiation of embryonic cells at critical points of development. Cell migration and organ construction are thereby impeded or wholly frustrated; proliferation, however, may not be greatly affected and may even be accelerated. Spemann [50] and others [51] have provided important clues to an understanding of the principles involved in faulty differentiation. In their studies on embryonic development they have shown that differentiation depends on two major factors, an intrinsic predisposition within cells to undergo specialization ("cell competence") and an inductive stimulus of chemical nature elaborated by certain previously specialized cells ("organizers"). The two factors are anatomically and functionally related. The primitive lens, for instance, is formed by differentiation of ectodermal cells in the head region in response to the inductive stimulus of the outgrowing optic vesicle of the forebrain. Ectodermal cells from other regions may also undergo lens differentiation if transplanted to the head region overlying the optic vesicle; conversely, transplantation of the optic vesicle will induce lens formation beneath the ectoderm of any region. The optic vesicle is said to be an "organizer" for lens formation. On the other hand, ectoderm normally intended for lens formation may be induced to undergo this change in the absence of the optic vesicle itself when subjected to the action of soluble substances extracted from the cells of the latter. According to Needham,[51] such substances are of definite chemical structure, being sterols homologous with estrogen and certain carcinogenic hydrocarbons. This is disputed by Spemann, who induced differentiation by extracts from many other parts of the

50. Spemann, H.: Embryonic Development and Induction, Silliman Memorial Lectures, New Haven, Conn., Yale University Press, 1938.

51. Needham, J.: New Advances in the Chemistry and Biology of Organized Growth, Proc. Roy. Soc. Med. **29**:1577 (Oct.) 1936.

embryo and even by simple acids (oleic acid, linoleic acid, nucleic acids and adenylic acid) but not by the ether-soluble nonhydrolyzable sterol fractions. The exact role of these acids is not clear, and they may serve merely as activators of some of the aforementioned sterols.

The harmonious interplay of these two factors, cell competence and organizer induction, insures complete integration between component cells of each growing organ. It seems likely that this principle of "double assurance" governs the growth of all embryonal structures. In other words, organizer influences depend not only on their own relative potency but on cell competence as well. The latter becomes less as differentiation approaches completion, and the fully differentiated cell may be said to be relatively refractory to organizer influence. Up to a certain stage of growth, however, a considerable measure of reversibility exists. Mesodermal tissue which has become notochord is capable of inducing the formation of a secondary neural plate in any part of the epidermis. Ectoderm may even become converted to notochordal mesoderm if transplanted into a powerful organizing "field," such as the region of the foregut; this mesoderm, in turn, becomes capable of inducing neural differentiation in any undifferentiated ectoderm. Entoderm, on the other hand, possesses an astonishing power of self-differentiation, in contrast to the high degree of induction susceptibility of ectoderm. The inductive power of mesoderm extends over a long period of development, far beyond the stage in which induction normally comes into play.[50] The theory of the immutability of the three germ layers is thus clearly superseded by the newer doctrine of differentiation by multiple organizers.

Transplantation of cells of the dorsal lip of the primordial blastopore induces the formation of an entire secondary, parasitic embryo in any portion of the host embryo. This capacity is to a lesser degree inherent in any part of the embryo, probably even in every cell, but its realization is suppressed by previously established organizers.[52] Injurious factors, such as heat, dryness and overripeness of the egg before fertilization, have been shown (in the frog) to interfere with the development of a normal hierarchy of organizers. In the chick similar effects have been produced by subjecting eggs to a subnormal temperature (90 F.) for the first forty-eight hours or more of incubation.[53] As a result, multiple competing organizers are released for simultaneous action on cellular substrates, and multiple malformations ensue (Witschi [54]).

52. Witschi, E.: Appearance of Accessory "Organizers" in Overripe Eggs of the Frog, Proc. Soc. Exper. Biol. & Med. **31**:419 (Jan.) 1934.

53. Smith, L. E. W.: Effect of Temperature on the Development of the Chick Embryo, Arch. Path. **28**:422 (Sept.) 1939.

54. Witschi, E.: (*a*) Experimentally Produced Neoplasms in the Frog, Proc. Soc. Exper. Biol. & Med. **27**:475 (March) 1930; (*b*) footnote 52.

By interference with the orderly sequence of embryonic organizers or by alteration of the spatial configuration of various induction centers, numerous malformations, such as ectopias and duplications (plus formations) have been produced experimentally. In addition, embryonal defects of many sorts have been seen in which the mechanism seems to have been a partial or complete suppression of embryonic induction (minus formations). Lehmann [55] found that the administration of chlorobutanol abolished specifically the induction of the lens by the optic vesicle and resulted in complete suppression of the lens or formation of an abnormally small lens. Lens formation was also blocked by the interception of a thin layer of mesodermal cells between the epidermis and the optic vesicle.[50] Lithium salts were found to inhibit the development of the embryonic notochord.[51] Hale described eyeless pigs born of vitamin A–deficient sows, and in similar experiments Zilva, Goldring, Drummond and Coward reported failure of limb formation. These and other examples prove that faulty differentiation occurs either through a diminution in cell competence in spite of normal inductive stimulus or through interference with the mechanism of organizer induction in normal cells. The operation of the principle of "double assurance" makes it probable that minor degrees of insufficiency of either factor do not interfere with differentiation.

Organizer Principles in Postembryonic Life and Their Bearing on the Tumor Problem.—Growth and individuation of cells do not come to an end with the birth of the individual. Regeneration of tissue after injury and to offset wear and tear involves not only a proliferation of "competent" reserve cells, usually only partly differentiated, but their maturation and architectural conformity in the preexisting "organization field." It is a fair assumption, then, that differentiation by organizer induction in the embryo represents only the initial phase of a biologic mechanism governing cell integration in the whole organism throughout life.

Little more than supposition can be made now in regard to the nature and situation of organizers within any given organ. They may be assumed to reside within certain of its cells, possibly in certain interstitial cells of the stroma (cf. the inductive power of mesoderm in the embryo), acting in the manner of an intrinsic hormone for the organ. Since the term organizer has nothing specific in its connotation, there is no reason to exclude from this concept the organizing action of certain extrinsic substances which govern cell differentiation in single organs, in physiologic groups of organs or in the body as a whole. In this broader view of organizers may therefore be included the definitive

55. Lehmann, cited by Spemann.[50]
56. Deleted by author.

endocrine organs of the body and possibly certain dietary factors. Taking an example at random, one may regard the genesis and entire life cycle of the corpus luteum as an organizer effect of certain hypophysial factors on the ovarian follicle. In a similar light one may regard the influence of vitamin C on the differentiation of mesenchymal cells into osteoblasts.[57] The term organizer as applied within the meaning of this paragraph actually refers to a category of substances, intrinsic or extrinsic to a given organ and known or unknown, which possess in common a hormone-like action on immature cells to induce their specific differentiation.

Organizers and Tumors: Investigators have not been laggard in attempting to draw comparisons between cell behavior of embryos and that of tumors. The newer work on embryonic organizers has therefore attracted increasing interest on the part of students of the tumor problem. At present, however, only certain suppositions may be hazarded, and these may soon be superseded as new facts come to light.

The present study of the pathogenesis of the tuberous sclerosis complex brings into the foreground the question of the organizer field as it concerns both abnormal development and malignancy. That the two are in some way related has long been suspected, and the dysontogenetic origin of certain cancers has been clearly established. The cancer cell may be described generally as the product of faulty differentiation of a proliferating cell, arising from reserve cells in the course of tissue regeneration. According to one view, the normal competence of such cells (i. e., their intrinsic genetic predisposition to complete their differentiation) becomes weakened by "carcinogenic" factors, chemical, physical, hormonal or infectious, so that these cells become refractory to organizer influences and may continue to proliferate excessively and eventually without limit. It has been suggested, therefore, that cancerization represents a true mutation. In addition, evidence has been accumulated to show that such anaplastic cells, potentially ancestral to cancer, are formed rather frequently (Fischer [58]), but for the most part remain latent, undergo delayed differentiation or die, so that actual cancers arise in only certain members of the population and usually rather late in life. So great is the diversity among individuals and species in susceptibility to cancer that investigators have turned their attention more and more from the cancer cell to the cancer host.

The principle of the organizer field, applied to problems of carcinogenesis, is concerned primarily with the state of the tissue in which cancer develops. Marked variations in "resistance" to neoplasm are

57. McLean, D. L.; Sheppard, M., and McHenry, E. W.: Tissue Changes in Ascorbic Acid Deficient Guinea-Pigs, Brit. J. Exper. Path. **20**:451 (Dec.) 1939.

58. Fischer, A.: The Theory of the Developmental Physiology of Malignant Tumors, Am. J. Cancer **31**:1 (Sept.) 1937.

encountered in man and in lower animals,[59] and susceptibility to spontaneous or induced neoplasm in animals may be markedly raised or lowered by a number of experimental procedures.[60] As previously stated, organizer effects are in a broad sense hormonal but are local or general in their origin and effect and may be transient or permanent. Since their nature is far too vague at present for any attempt at definite characterization, they are best understood, perhaps, by inference from conditions in which, presumably, their action is impaired. The following general factors may be concerned: (*a*) the stroma of tissues, (*b*) the age of the organism, (*c*) the functional state of the nerve supply of the tissues and (*d*) preexisting anomalies of development.

(*a*) The stromal factor in malignancy. The possibility was mentioned previously that the site of organizer production in mature organs may be the cellular constituents of their stroma. It should be recalled that the inductive power of embryonic mesoderm has been shown to extend over a long period of development, and its continuation in postembryonic life may therefore be reasonably sought in such mesodermal derivatives as the stroma of organs, the spleen and various types of connective tissue. Morton and Beers (1935 [61]) reported the demonstration in normal human connective tissue of a tumor growth-inhibiting factor. Baker,[62] moreover, attempted to retard the progress of cancer in

59. Warren, S., and Gates, O.: Spontaneous and Induced Tumors of the Guinea Pig, Cancer Research **1**:65 (Jan.) 1941.

60. (*a*) Mori, K., and Nakahara, W.: Effect of Liver Feeding on the Production of Malignant Tumors by Injections of Carcinogenic Substances, Gann **34**:48 (April) 1940; abstracted, Cancer Research **1**:77 (Jan.) 1941. (*b*) Sugiura, K., and Rhoads, C. P.: Experimental Liver Cancer in Rats and Its Inhibition by Rice-Bran Extract, Yeast, and Yeast Extract, ibid. **1**:3 (Jan.) 1941. (*c*) Gross, L.: Experimental Anti-Cancerous Immunity, J. Mt. Sinai Hosp. **6**:146 (Sept.-Oct.) 1939. (*d*) MacFadyen, D. A.; Sturm, E., and Murphy, J. B.: Inhibition of Transplantable Mouse Tumor Growths by Tissue Extracts and Their Protein Fractions, J. Exper. Med. **70**:475 (Nov.) 1939. (*e*) Maisin, J., and Pourbaix, Y.: Growth-Promoting and Growth-Inhibiting Substances from Normal Organs, Am. J. Cancer **24**:357 (June) 1935. (*f*) Lewisohn, R.; Leuchtenberger, R., and Laszlo, D.: Spleen Extract in the Treatment of Transplanted and Spontaneous Malignant Tumors in Mice, Surg., Gynec. & Obst. **71**:274 (Sept.) 1940. (*g*) Cheever, F. S., and Janeway, C. A.: Immunity Induced Against the Brown-Pearce Carcinoma, Cancer Research **1**:23 (Jan.) 1941. (*h*) Russell, B. R. G.: The Nature of Resistance to the Inoculation of Cancer, Scient. Rep. Invest. Imp. Cancer Research Fund **3**:341, 1908. (*i*) Salter, W. T.; Nathanson, I. T., and Wilson, H.: Experimentally Induced Benignancy of Neoplasm: V. The Influence of Hormones on the Host's Resistance to Implanted Neoplasm, Cancer Research **1**:60 (Jan.) 1941.

61. Morton, J. J., and Beers, D. N.: The Demonstration of a Tumor Growth-Inhibiting Factor from Normal Human Connective Tissue, J. Exper. Med. **61**:59 (Jan.) 1935.

62. Baker, H. S.: The Treatment of Cancer with Connective Tissue Extracts, Lancet **2**:643 (Sept. 16) 1933.

human patients with connective tissue extracts and reported a certain measure of success. The recent work of Lewisohn and his co-workers [60] on the use of spleen extracts in tumor-bearing animals arouses interest in the spleen as a factor of particular importance to the organism as a whole as a specialized source of the same (or similar) organizer principles as found in the embryonal mesoderm.

Indirect evidence of the relation of the organizer principle to the stroma of organs is obtained from pathologic observations which indicate a decided predisposition to malignancy in tissues which have undergone marked fibrosis. The best known examples are seen in instances of carcinoma of the breast occurring in fibrous mastopathies, carcinoma of the liver in cirrhosis and carcinoma of the skin in ancient scars of cutaneous burns. Since the nature of the organizer within mature organs is practically unknown, it is impossible to formulate any definite idea of the mechanism by which its effect is frustrated in the presence of marked stromal fibrosis. It would be interesting, none the less, to compare the tumor-inhibiting action of extracts of normal organs with those of organs in which the stroma has become replaced by indifferent scar tissue.

(*b*) The age factor. Aging is another factor predisposing to malignancy. In the later decades of life, organizer activity may be said a priori to have begun to wane along with other tissue functions and even cells of a relatively low order of anaplasia are liberated from restraining influences. For the same reason, numerous hamartoma-like changes also take place, such as the development of hyperkeratoses, soft fibromas and capillary angiomas of the skin, also benign adenomatous polypi and papillomas of mucous membranes. In the embryo, in infancy and in childhood, on the other hand, organizer activity may be presumed to be of such potency that only under certain special conditions do tumors succeed in gaining headway at all. Malignant tumors in infants and children are of embryonal type, often of complex teratoid structure, and are probably of dysontogenetic origin in the great majority of cases, even though they may not appear until some years after birth.[63] They progress rapidly and metastasize widely. Lymphocytic reaction and hyalinization of stroma are absent to a notable degree.[63] Certain instances have been recorded in human infants in which highly malignant tumors (neuroblastomas) have undergone spontaneous regression, with either complete cure or partial remission into a much more benign type of growth.[64] It is an inviting thought that in young individuals organizer influences appear to be sufficiently powerful to succeed at times in regaining dominance over the cells of a vigorous malignant growth. The potency of

63. Dargeon, H. W.: Cancer in Childhood, and a Discussion of Certain Benign Tumors, St. Louis, C. V. Mosby Company, 1940, pp. 17-18.

64. Wells, H. G.: Occurrence and Significance of Congenital Malignant Neoplasms, Arch. Path. **30**:535 (Aug.) 1940.

organizers in youth probably also explains the delay in clinical appearance of such hereditary or familial hamartomatous diseases as adenomatosis of the colon [65] and multiple neurofibromatosis,[66] as well as the tuberous sclerosis complex.[20] Several years often elapse before the earliest manifestations of these disorders appear, although they are undoubtedly determined in the tissues long before birth.

The possibility of influencing favorably the resistance to malignancy of tumor-bearing animals by treatment with embryo emulsion or embryo grafts was reported as far back as 1908 (Russell [60b] and confirmed subsequently.[67] Recent experiments on mice with substances isolated from the urine of a 4 year old girl seem to have yielded similar results.[68]

(*c*) Innervation of tissues. Another factor of great importance in determining organizer influence within tissues is the presence of nerve supply. The evidence for the close relation between the nervous system and local organizer function in the tissues lies in the sphere of growth and development, as well as in that of tumor formation.

A close relation is known to exist between the nervous system and organizer activity in the embryo, and it is difficult to imagine that this relation does not persevere in some form throughout the life of the individual. Its importance in lower forms has been clearly proved in the matter of regeneration. In earthworms and other annelids regeneration of highly individualized segments, such as the head, generally requires the presence of the main nerve cord.[69] Regeneration of a claw or leg in crabs and other crustaceans has recently been shown also to depend on a similar type of nervous integration. Since a mammal cannot reproduce a limb or similar unit of structure, the importance of the nervous system in regeneration is much less apparent. As far as I have found in the available literature, the factor of innervation has not yet been studied in regenerating tissues of mammals. It is theoretically conceivable that denervated tissues would show a tendency to over-

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65. As a rule in families affected by adenomatosis of the colon the tumors begin to develop at about the age of 20 years, and malignant changes may be expected about fifteen years later (Lockhart-Mummery, J. P., and Dukes, C. E.: Familial Adenomatosis of Colon and Rectum: Its Relationship to Cancer, Lancet **2**:586 [Sept. 9] 1939).

66. Sharpe, J. C., and Young, R. H.: Recklinghausen's Neurofibromatosis: Clinical Manifestations in Thirty-One Cases, Arch. Int. Med. **59**:299 (Feb.) 1937.

67. Murphy, J. B., and Sturm, E.: A Factor from Normal Tissues Inhibiting Tumor Growth, J. Exper. Med. **60**:293 (Sept.) 1934; The Effect of a Growth-Retarding Factor from Normal Tissues on Spontaneous Cancer of Mice, ibid. **60**: 305 (Sept.) 1934.

68. Dobrovolskaïa-Zavadskaïa, N., and Zephiroff, P.: Eket des produits d'origine épiphysaire et d'origine hépatique sur la croissance des tumeurs chez la souris, Compt. rend. Soc. de biol. **134**:60, 1940.

69. Hyman, L. H.: Aspects of Regeneration in Annelids, in Biological Symposia, Lancaster, Pa., Jaques Cattell Press, 1941, vol. 2, p. 241.

regeneration, with production of hypertrophies. This should be tested experimentally. Schwannomas ("amputation neuromas"), which form in nerve stumps shortly after nerve section, can be induced to undergo prolonged growth if their normal reinnervation is prevented by avulsion of the nerve root (Masson [70]). Repeated section of the distal nerve stump is followed by growth of these experimental schwannomas to enormous size, with invasion of the surrounding muscles.

Masson has stressed the close interrelation between carcinoid tumors of the intestinal tract and neuromatous changes in the periglandular nerve plexus of the intestinal mucosa.[71] The frequent occurrence of carcinoid tumors in obliterated appendixes has drawn attention to their association with strangulation of nerve filaments and the consequent development of amputation neuromas. The carcinoid cells appear to arise from argentaffin cells of the intestinal epithelium [71b] and to gain a certain measure of autonomy of growth, possibly as a consequence of the perversion of nerve function. With rare exceptions these carcinoids are benign stationary growths; in the rare cases in which they exhibit invasive tendencies, the metastases are, with few exceptions, slow growing or stationary and show few of the features of aggressive malignancy.[72]

Congenital malignant neoplasms constitute a separate category from other tumors, according to Wells,[64] possibly by virtue of the peculiar metabolic properties of the tissues of their origin. Nearly all such tumors are sarcomas, and of these a large proportion are of neurogenic origin. Evidence is found that congenital maldevelopments of nerve tissues occur frequently, and these apparently may disappear, develop into malignant tumors, develop into benign tumors or even become malignant and then change in whole or in part into nonmalignant tissue. A large proportion of all neurogenic malignant tumors derived from the sympathetic nervous system and from the retina are present at birth, while congenital neoplasms of the brain and of the peripheral nerves are extremely rare (Wells [64]).

Recklinghausen's neurofibromatosis is marked by multiple benign tumors of the skin and internal organs in the course of peripheral nerves.

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70. Masson, P.: Experimental and Spontaneous Schwannomas (Peripheral Gliomas), Am. J. Path. **8**:367 and 389 (July) 1932.

71. Masson, P.: (*a*) Neural Proliferations in the Vermiform Appendix, in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 3, sect. 25, p. 1095; (*b*) Carcinoids (Argentaffin-Cell Tumors) and Nerve Hyperplasia of the Appendicular Mucosa, Am. J. Path. **4**:181 (May) 1928.

72. Terplan, K.; Weintraub, D., and Wolf, N. J.: Stationary Metastasizing Carcinoid of the Ileocecal Valve, Arch. Path. **30**:1155 (Nov.) 1940. Ariel, I. M.: Argentaffin (Carcinoid) Tumors of the Small Intestine: Report of Eleven Cases and Review of the Literature, ibid. **27**:25 (Jan.) 1939. Bailey, O. T.: Argentaffinomas of the Gastro-Intestinal Tract, Benign and Malignant, ibid. **18**:843 (Dec.) 1934.

In addition, the disease exhibits peculiarities of development of isolated regions of the body, skeletal and visceral, which take the form of localized overgrowth or gigantism. It is probable that these are etiologically related to disturbances in innervation.

Timme (1913) [73] succeeded in producing marked hypertrophy of the stomach and colon experimentally in cats by partial interruption of both vagus nerves through constricting ligatures. Secretory, as well as motor, elements were increased in size and number; function, however, was greatly depressed. These experiments were cited by Scherer [74] in a discussion of the marked hypertrophy of the esophagus and stomach and of the giant appendix in the case of neurofibromatosis which he reported in which the vagus nerves were affected.

The latter disease presents a number of general parallels to the tuberous sclerosis complex as well, and these will be discussed later. One element in the disease which is of particular pertinence at this point of discussion is the frequent association of abnormalities of peripheral nerves with the development of sarcomas which may be highly malignant. The incidence of neurogenic sarcoma is said to range between 8 and 13 per cent of all cases.[75]

Ewing [76] cited Rindfleisch's opinion and the experiments of Remond and of Pearce and van Allen as evidence for the fact that defective nervous control is one of the factors predisposing to exaggerated growth of tissues and tumor formation.

There is much room for thought in regard to a possible mechanism by which nervous influences achieve an organizer effect on tissues. One may predicate, perhaps, a secretagogue-like nervous stimulation of specialized stromal cells subserving organizer function within organs. The effect, however, may be a direct one on all cells. The old concept of "trophic" nervous influence has an insecure factual basis but may possibly support analysis through this newer approach.

(*d*) Maldevelopment and the predisposition to tumor. One further factor involving organizer action in relation to neoplasia is the presence of developmental anomaly. The predisposition to malignancy of persistent embryonal remnants and malformations is an established principle.[14] Malignant tumors have been found in experimentally produced embryonic monsters as well; these tumors continue to grow and invade after transplantation into a normal animal.[54a] In the light of the researches already mentioned, it is possible now to trace a common background

73. Timme, W.: Experimental Studies on the Nervous Mechanism in the Production of Hyperplasia, J. Nerv. & Ment. Dis. **40**:311, 1913.

74. Scherer, H. J.: Zur Frage des Zusammenhanges zwischen Neurofibromatose (Recklinghausen) und umschriebenem Riesenwuchs, Virchows Arch. f. path. Anat. **289**:127, 1933.

75. (*a*) Kaufmann,[4] vol. 2, p. 1568. (*b*) Sharpe and Young.[60]

76. Ewing,[14] p. 107.

for both developmental anomalies and tumors related to them in a perversion of organizers. The primary effect is a frustration of normal cell integration in certain areas, resulting in teratoid formations and embryonal rests; thereafter the local deficiency in organizers persists, and newly proliferated cells in later life are deprived of the normal impetus to maturation in these areas. Such cell districts may be said therefore to possess an increased susceptibility (or diminished "refractoriness") to the development of tumors. Thus the origin of certain malignant tumors in embryonal rests, as postulated by the Cohnheim-Ribbert theory,[14] is made consistent with the principle of defective induction of differentiation through faulty organizer mechanisms.

The "transition" of benign into malignant neoplasm is probably identical in principle with the theory of origin of cancer in embryonal rests. There is a growing tendency to separate benign tumors from the general category of neoplasms and to group them with growth anomalies. As Reimann [77] stated: "Benign growths have an organization, while malignant ones do not, and cellular differentiation in benign growths is quite different anatomically (and physiologically by presumption, for many reasons) from that in malignant growths." This is essentially the characterization of hamartoma,[12] which is a designation applied to many so-called benign tumors of organoid structure and, as already implied in this paper, is the underlying basis for the pathogenesis of the tuberous sclerosis complex. In a broad sense, therefore, predisposition to cancer may be regarded fundamentally as a fault in organizer action which permits cancer cells to spring into being, either directly from "irritated" reserve cells of mature tissues or in the course of a stepwise degradation of organizer potency, the first manifestation of which is an embryonal tissue defect (hamartia), succeeded in turn by a benign tumor (hamartoma) and eventually by a malignant tumor of slow or rapid evolution (hamartoblastoma).

The sequence of change from hamartia to hamartoma or to hamartoblastoma is probably not restricted to embryonic cells but may arise equally well in reserve cells of normal tissues at any period of mature life in the event of a defective process of regeneration. It is possible in this manner to explain the development of the hamartomas of adults, especially of old persons (fibromas, nevi, angiomas, etc.), as well as malignant mixed tumors of the testicle (hamarblastomas).

The Organizer Principle in Relation to the Pathogenesis of the Tuberous Sclerosis Syndrome and Its Neoplastic Complications.—With the foregoing generalizations in mind, one can quickly recognize in the tuberous sclerosis complex a particularly fortunate natural experiment

77. Reimann, S. P., and Toennies, G.: Reaction of Mouse Skin to Various Reduced and Partially Oxidized Sulfur Compounds, Arch. Path. **29**:175 (Feb.) 1940.

for the testing of several implications of the organizer principle in human biology. As analyzed at present, the disease has its basis in a disseminated tissue malformation complicated by neoplastic overgrowths, chiefly in the brain. The basis for the ontogenetic fault may now be laid to a faulty sequence of interaction of organizers and cells rather than to cell defects alone, inasmuch as one of the truly important characteristics of the disease is its apparent inception in numerous widely scattered areas in a single interval of embryonal time. This fact reminds one of the manifold malformations ensuing in the frog embryo as a result of delayed fertilization [54] or in the chick embryo as a result of subnormal incubation temperature in the early hours after development has begun.[53] Furthermore, all gradations between normal and markedly abnormal cells have been shown to exist in affected areas, and the cytologic line of demarcation is often difficult to determine.

The organizer principle was utilized by Worster-Drought (1937) [78] in an analysis of the possible pathogenetic mechanism of several related syndromes of multiple tumor formation (Lindau's disease, tuberous sclerosis, neurofibromatosis, etc.) and, in particular, of 2 cases of multiple meningeal and perineural tumors (Wishart type of central neurofibromatosis). The implication of his analysis was that the mechanism of these disturbances was to be found in an excessive or unregulated organizer activity leading to multiple tumor or tumor-like formations. The importance of this generalization in aiding the interpretation of these syndromes cannot be overestimated.

Nevertheless, it is my belief that the primary emphasis should be placed not on excessive and indiscriminate organizer activity but on its frustration, either through intervening factors or through intrinsic defects in the cells themselves. Since, as already mentioned, the separate lesions of the disease exhibit throughout the body an unmistakable parallelism in their general features and in their chronology, a single biologic "incident" affecting many areas simultaneously is suggested. It is much more likely, for this reason, that the basic element in pathogenesis involves a disturbance in timing whereby the interaction of organizers and cells is disturbed at a certain crucial moment. Once that moment has passed, the organizers for specific inductions are superseded by new organizers and the cells of the affected regions are stranded, so to speak, in a condition of permanent embryonicity. In the hamartial foci which constitute the disease, the cells retain throughout life many of their original embryonal characters, exhibiting a singular inertia in the presence of later maturational influences. The appearance

78. Worster-Drought, C.; Dickson, W. E. C., and McMenemey, W. H.: Multiple Meningeal and Perineural Tumours with Analogous Changes in the Glia and Ependyma (Neurofibroblastomatosis), with Report of Two Cases, Brain **60**:85 (March) 1937.

of these lesions suggests, therefore, inhibition (minus formation) rather than stimulation (plus formation). The lesions, it is true, possess an imperfect architecture and in that respect simulate neoplasms. Nevertheless an organoid constitution is usually evident. The refractoriness to normal host influences is more marked in cells destined primarily for function rather than in those destined for support. The former elements may be scanty and poorly formed, as the abortive ganglion cells of the cerebral foci or the abortive tubules of the renal foci. The latter elements (mesenchyme, vasculature, glia) accordingly dominate the picture and produce the tumor-like bulkiness and nodularity of the lesions.

The question of malignancy in this disease is properly joined with that of its hamartial nature. In both respects the heart of the problem is the defective activity of local organizers. From this standpoint, the factor of dysontogenesis, instead of predisposing to malignancy, as in cancer in general, seems to result in a relative antagonism to malignancy. It is difficult to resolve this apparent inconsistency on theoretic grounds, unless by the supposition that the abnormal cells, despite their embryonal structure, have nevertheless undergone as complete a differentiation as their limited competence permits and are thus incapable of proliferation endlessly in the manner of tumor cells. By the same token it is implied that these cells continue to respond to organizers within the master tissues after allowance is made for the gap in the organizer series to which they owe their malformation. The disorganizing factor of disturbed innervation, while much less important for the body as a whole than in multiple neurofibromatosis, is nevertheless prominent in the tuberous foci in the brain. As previously stated, there is a high incidence of malignant mixed tumors of the brain under such circumstances, and these are often multiple. In the other foci of the disease, malignancy is rare and is apparently restricted to the renal nodules. In their slow growth and restricted metastases their behavior is much more like that of carcinoids of the appendix than of malignant mixed tumors of the Wilms type.

TERMINOLOGY AND CLASSIFICATION OF THE TUBEROUS SCLEROSIS COMPLEX

These several considerations offer new possibilities in terminology and classification of the tuberous sclerosis complex. The disease may now be accepted, according to most studies, as a widespread developmental anomaly, often hereditary, in which the primary abnormality lies in the faulty differentiation of tissues rather than of body units (although the latter may be included). The individual lesions present the characters of hamartia or of hamartomas, regardless of their situation.

Because other organs are involved simultaneously with the brain, the term tuberous sclerosis, which refers only to the cerebral lesions, should be reserved for these and should not be employed to designate the syndrome as a whole. For the latter the term disseminated hamartiosis (Bourneville type) may be more suitable.

OTHER TYPES OF DISSEMINATED HAMARTIOSIS

Multiple Neurofibromatosis.—Reference should be made again to the numerous points of resemblance between the tuberous sclerosis complex and Recklinghausen's multiple neurofibromatosis. There is much discussion on this point,[45] and not a few writers regard the two diseases as related,[79] if not identical, in principle.[80]

This viewpoint is not shared by all, however. The common impression of the pathogenesis of the cutaneous and visceral nodules of neurofibromatosis is based on the studies of Verocay, who considered them benign neoplasms of the nerve sheaths. According to a later view, they are basically of more primitive origin (undifferentiated neuroepithelium[75a]) and originally contain both nerve cell and supporting cell elements, with the latter predominating and crowding out the former.

The point has also been raised that these nodules are hamartomas, i. e. malformations, which arise out of these primordial nerve elements,[81] and are therefore not true neoplasms. A number of gross malformations of more obvious character have also been described in this disease (epispadias, cryptorchidism, macroglossia, polythelia, bony and skeletal defects, infantilism),[45] adding much weight to the dysontogenetic interpretation. The high incidence of neurogenic sarcoma in this disease bears out notably the importance of two of the factors previously stated to interfere with organizer function—abnormal innervation and maldevelopment. The superimposition of both factors must a priori be expected to conduce to overgrowth and to neoplasia.

The palisaded nodules of the skin are classifiable as hamartomas of the Wagner-Meissner tactile corpuscles (Masson[70]) and the sarcomas of the soft parts as hamartoblastomas (fibrosarcoma, neurosarcoma) of the nerve sheaths. The regional hypertrophies associated with this disease

79. Globus, J. H.: Malformations in the Central Nervous System, in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 3, p. 1150.

80. (*a*) Bielschowsky, M., and Gallus: Ueber tuberöse Sklerose, J. f. Psychol. u. Neurol. (Ergnzngshft.) **20**:1, 1913. (*b*) Orzechowski, K., and Nowicki, W.: Zur Pathogenese und pathologischen Anatomie der multiplen Neurofibromatose und der Sklerosis tuberosa (Neurofibromatosis universalis), Ztschr. f. d. ges. Neurol. u. Psychiat. **11**:237, 1912. (*c*) Yakovlev and Guthrie.[28a]

81. Sharpe and Young.[68] Masson.[70]

may be primary malformations (Pick [82]) or may be manifestations of the neurodystrophic deviation of organizer function. Such hypertrophies prove horribly disfiguring when they occur as localized elephantiasis of the face or of a limb.[83] Gigantism of the esophagus and stomach and segmental hypertrophy of the intestine [84] or appendix [74] have been recorded in association with changes in the vagus nerves and submucosal nerve plexuses.[85] Chromaffinoma of the adrenal glands [86] may even represent the effect of homologous changes in the sympathetic nerves and thereby furnish a close counterpart of the carcinoids of the appendix. Meningeal tumors, small foci of cortical gliosis and glioma-like tumors of the brain have been recorded in cases of neurofibromatosis and have given rise to the concept of central forms of the disease.[87] Defects in intelligence and infantilism have also been observed clinically.[66] In a few instances of multiple neurofibromatosis fragments of the tuberous sclerosis complex have also been noted, e. g., adenoma sebaceum [30a] and phacoma of the retina,[35] but otherwise the hamartial tendency is confined to the structures already described.

Lindau's Disease and Related Syndromes.—In an adjacent category may be placed the complex known as Lindau's disease.[88] This syndrome presents a distinctive type of retinal angiomatosis (von Hippel's disease) with associated angiomas of the cerebellum, brain stem and spinal cord. The cerebellar angiomas may become cystic and are prone to recurrent hemorrhage, which may cause sudden death. Cysts of the kidney and pancreas are frequent, as are also adenomas or "hypernephromas" of the kidney. Like tuberous sclerosis and neurofibromatosis, Lindau's disease occurs as a mendelian dominant and is thus both hereditary and familial. The angiomas of this disease may be regarded as hamartomas of vascular type occurring within a restricted organ distribution.

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82. Pick, L.: Ueber Neurofibromatose und partiellen Riesenwuchs, mit mesenterialer Neurofibromatose insbesondere über die sektorenförmige Kombination von wahrem partiellen Riesenwuchs des Darmes, Beitr. z. path. Anat. u. z. allg. Path. **71**:560, 1923.

83. Ewing,[14] p. 164. Sharpe and Young.[63]

84. Lotz, A.: Der partielle Riesenwuchs mit besonderer Berücksichtigung des sogenannten sekundären, eine pathologisch-anatomische Untersuchung, Inaug. Dissert., Berlin, G. Schade, 1914.

85. Scherer.[74] Pick.[82] Lotz.[84]

86. Rosenthal, D. B., and Willis, R. A.: The Association of Chromaffin Tumours with Neurofibromatosis, J. Path. & Bact. **42**:599 (May) 1936.

87. Worster-Drought, Dickson and McMenemy.[78] Footnote 80*a* and *b*.

88. Lindau, A.: Studien über Kleinhirncysten: Bau, Pathogenese, und Beziehungen zur Angiomatosis retinae, Acta path. et microbiol. Scandinav., 1926, supp. 1, p. 1. Cushing and Bailey.[7a]

It is not unlikely that heredofamilial angiomatosis of the skin and mucosa (Osler's disease [89]) is based on the same dysontogenetic principles. Another complex has been described, encephalotrigeminal angiomatosis, which suggests some of the general features of tuberous sclerosis.[90] Its outstanding features are naevus vasculosus of the face in the distribution of the fifth cranial nerve, slight angiomatosis of the pia mater and aplasia, sclerosis and calcification of parts of the cerebral cortex. Clinically one notes epilepsy, mental retardation and slight spastic hemiplegia contralateral to the facial nevus.

Congenital polycystic disease should also be grouped among the disseminated hamartioses. In its histopathologic features can be clearly recognized a diffuse hamartial disturbance affecting the duct pattern of one or more organs, leading to the formation of cysts. Although its most frequent manifestation is in the kidneys, it involves the liver frequently [90a] and less often the pancreas and other organs. It is a heredofamilial disorder and is frequently associated with other developmental anomalies (harelip, meningocele, polydactyly, spina bifida).

Irregular Types of Hamartiosis.—A number of individual cases are on record of a disseminated hamartial type of maldevelopment in which classification among well defined syndromes cannot be made. Three cases of congenital muscular hypertrophy, extrapyramidal motor disturbances and mental deficiency were reported by de Lange.[91] Peculiar changes in the convolutions (polygyria, microgyria), partial underdevelopment of certain parts of the brain and overdevelopment of others and widespread microscopic porencephaly were among the features found at autopsy in 1 case. Weil [92] reported a case of familial megalencephaly in a boy aged 7 whose brain weighed 1,856 Gm. In addition to the interstitial hypertrophy of the cerebrum (glial hypertrophy) the brain stem and cerebellum presented diffuse "glioblastomatosis." The precentral area was poorly developed. The skeletal muscles were also underdeveloped. The medulla of the adrenal glands was practically devoid of chromaffin tissue. Stewart reported a case of muscular dystrophy with feeblemindedness, pronounced vagotonia, hypertrophy of

89. Madden, J. F.: Generalized Angiomatosis (Telangiectasia), J. A. M. A. **102:** 442 (Feb. 10) 1934.

90. Krabbe, K. H.: Facial and Meningeal Angiomatosis Associated with Calcifications of the Brain Cortex, Arch. Neurol. & Psychiat. **32:**737 (Oct.) 1934. Yakovlev and Guthrie.[28a]

90a. Moolten, S. E.: Congenital Anomalies of the Smallest Bile Ducts, to be published.

91. de Lange, C.: Congenital Hypertrophy of the Muscles, Extrapyramidal Motor Disturbances and Mental Deficiency, Am. J. Dis. Child. **48:**243 (Aug.) 1934.

92. Weil, A.: Megalencephaly with Diffuse Glioblastomatosis of the Brain Stem and the Cerebellum, Arch. Neurol. & Psychiat. **30:**795 (Oct.) 1933.

the salivary glands and peculiar warty nodules of glial hyperplasia in the cerebral cortex which suggested an unusual type of "primary gliosis" rather than an abortive form of tuberous sclerosis.[93] These and similar conditions are perhaps conveniently grouped as disseminated hamartioses of irregular type. Again, as in the tuberous sclerosis complex, the most noteworthy error of development is found in the nervous system.

SUMMARY

A case of the tuberous sclerosis complex in a girl aged 20 is described. The disease was first brought to light clinically by hemorrhage from a renal hamartoma. The latter was of striking appearance but typical histologic structure and contained a large angiomatous component and a caliceal anomaly. The patient also presented adenoma sebaceum, phacoma of the retina and clinical evidences of typical cerebral involvement. The course of her later illness resembled that of a tumor involving the brain stem in the region of the left corpus quadrigeminum and aqueduct of Sylvius. Left ophthalmoplegia with Argyll Robertson pupil were present, also papilledema and other evidences of internal hydrocephalus.

The tuberous sclerosis complex may be epitomized as a disseminated disease, often heredofamilial in type, characterized by numerous defects in tissue combination (hamartia). The brain, heart, retina, kidneys and skin are conspicuously affected. The individual lesions range in size from microscopic foci (hamartial "germs") to visible nodules (hamartomas) having a superficial resemblance to neoplasms. Developmental defects of a more serious type, such as cardiac or renal anomaly and so-called stigmas of degeneracy, often coexist and corroborate the view that the disease has its inception in the earliest stages of embryonal development.

Malignant neoplasms supervene with extreme rarity in the somatic foci of the disease and are slow growing and produce few metastases. In the cerebral foci, however, such tumors appear to occur much more frequently, taking the form of a malignant mixed tumor (neurospongioblastoma), which is often multiple.

The concept of a defective mechanism of induction by embryonic organizers (Spemann) offers a logical explanation of the pathogenesis of this disease and of the accompanying developmental defects. There is much reason to believe that the same principle underlies the later complication of malignancy, especially in the brain. In a study of this disease as a type it is possible to derive certain generalizations which have application to problems of more general importance, chiefly the genesis of congenital dysplasia and the predisposition to tumor. The

93. Stewart, R. M.: An Unusual Type of Cortical Gliosis, J. Neurol. & Psychopath. **15**:160 (Oct.) 1934.

embryonic systems of organizer action are probably the initial phase of certain basic mechanisms of cellular integration which normally govern differentiation and maturation of proliferating cells throughout life. Excessive fibrosis, aging and preexisting malformation are factors which evidently impair such control and thereby predispose to malignancy. Neurotrophic influences are apparently also important in the maintenance of the organizer field, and the predisposition to brain tumor in tuberous sclerosis is probably only one of many instances in which defects in neural integration underlie the predisposition both to anomalous overgrowth and to neoplasia.

The similarities between the tuberous sclerosis complex, multiple neurofibromatosis, encephalotrigeminal angiomatosis, Lindau's disease and related syndromes are sufficient to establish them all as forms of disseminated hamartiosis. The latter term may be employed as a general category of classification for syndromes such as these in order to emphasize their non-neurologic features.

ACUTE BACILLARY DYSENTERY

A CLINICOPATHOLOGIC STUDY OF TWO HUNDRED AND SIXTY-THREE CONSECUTIVE CASES

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During the past eleven years, from Jan. 1, 1930, to Jan. 1, 1941, 263 cases of acute bacillary dysentery have been recorded at the Gorgas Hospital. In all cases this diagnosis was confirmed by stool cultures positive for the causative organism. It was felt that a careful study of this group of cases might be of some value in a better understanding of the disease. No attempt at an exhaustive review of the literature has been made.

Felsen [1] stated in 1939 that there was a rising incidence of bacillary dysentery in the United States, the reported figures for 1937 being approximately sixteen times those for 1933. He also pointed out that as the reported cases of bacillary dysentery increase there is a corresponding decline in the number of cases of unclassified "diarrhea and enteritis." During the period covered by this survey 3,556 cases of unclassified "diarrhea and enteritis" have been recorded at the Gorgas Hospital, while only 263 cases of bacillary dysentery and 240 cases of amebic dysentery have been reported.

INCIDENCE

The cases were distributed throughout the period as shown in table 1. There was a rather marked rise in the number of reported cases beginning in 1935. The incidence for 1930 is possibly somewhat misleading because of the occurrence of an epidemic during that year, which will be referred to later. There was no significant seasonal variation. The age distribution is shown in chart 1. The number of patients less than 1 year old was small and peaks were reached between the ages of 1 and 5, and 21 and 25. There was a significant difference in sex

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1. Felsen, J.: Newer Concepts of Intestinal Infection, J. A. M. A. **112**:46-49 (Jan. 7) 1939.

incidence, with males outnumbering females almost 2:1 (table 2). The relation of color and nationality to the disease was of less importance.

In 26 of these 263 cases, such obvious complicating and wholly distinct disease processes were apparent at the time of admission to the hospital that those cases were not included in the clinical study. This leaves 237 cases which were so studied.

TABLE 1.—*Date of Onset*

Year	No. of Cases	Month	No. of Cases
1930	33	January	26
1931	3	February	53
1932	8	March	29
1933	3	April	12
1934	3	May	13
1935	16	June	22
1936	45	July	17
1937	28	August	10
1938	52	September	19
1939	44	October	18
1940	28	November	22
		December	22

TABLE 2.—*Relation of Sex, Color and Nationality to Incidence of Acute Bacillary Dysentery*

	No. of Cases	Percentage
Sex		
Male	170	64.7
Female	93	35.3
Color		
Negro	149	56.6
Native white	110	41.8
Foreign white	4	1.5
Nationality		
Panamanian	100	38.0
American	93	35.3
West Indian	55	20.9
Others	15	5.7

NUMBER OF DAYS BETWEEN ONSET OF SYMPTOMS AND ADMISSION TO HOSPITAL

The period of incubation could not be determined, as in no case was the exact time of exposure known. Woolpert and associates [2] reported a case in which the disease occurred in a laboratory worker as a result of accidental inoculation and in which the incubation period

2. Woolpert, O. C.; Marsh, H. F., and Yaw, O. F.: Bacillary Dysentery Accidentally Incurred in the Laboratory, J. A. M. A. **113**:753-754 (Aug. 26) 1939.

could be accurately determined as forty-eight hours. Table 3 shows the number of days during which symptoms had been present before the patient entered the hospital; 33 per cent entered during the first twenty-four hours of disease, while 94 per cent entered during the first seven days.

SYMPTOMS AND PHYSICAL FINDINGS

The symptoms complained of are recorded in chart 2. The word dysentery carries with it an implication of diarrhea. Actually this complaint was elicited in only 80 per cent of all cases, and in 4 per cent such a manifestation was specifically denied. Smith [3] studied an epidemic of Sonne dysentery, occurring in England in 1931, in which diarrhea was present in only 57 per cent of the cases. The number of stools varied from one to sixty per day. The presence of gross blood in the stool was observed and reported by the patient in 48 per cent of

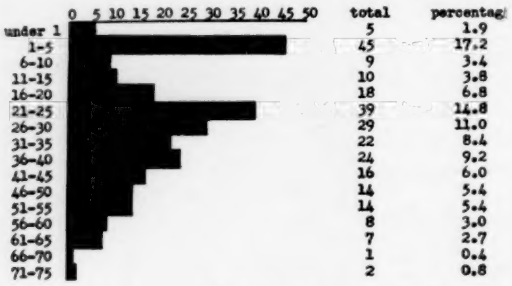

Chart 1.—Age incidence of 263 cases of acute bacillary dysentery.

TABLE 3.—*Interval Between Onset of Symptoms and Admission to Hospital*

No. of Days	No. of Cases
1	74
2	26
3	38
4	27
5	22
6	10
7	11
8	4
9	1
10	3
14	3
Over 14	4

the cases. Abdominal pain, which was commonly present, manifested itself as a crampy pain occurring anywhere along the course of the

3. Smith, R. E.: A Clinical Description of Epidemic Sonne Dysentery, Lancet **2**:925-927 (Oct. 24) 1931.

colon and was poorly localized. Convulsions were reported by 8 patients, all of whom were children with high fever.

The only physical finding of importance was abdominal tenderness, which varied from slight to rather marked. This was present in 52 per cent of all cases. In some it was accompanied by voluntary spasm of the abdominal wall, but in no case was true rigidity noted. There was some tendency to localization in the lower quadrants, but in most cases it was present diffusely, and there was no significant difference between the two sides of the abdomen. Abdominal distention was encountered only rarely and was always mild.

No attempt was made to classify these cases according to such arbitrarily designated types of disease as "meningitic," "pneumonic" or "appendicular," as it was felt that such terms do not signify sharply defined clinical entities. It will be understood that the clinical picture does vary somewhat from case to case, as is true in all diseases, depending on the predominance of one or more of the various symptoms and signs.

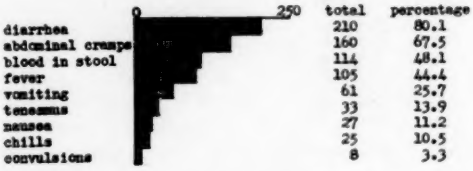

Chart 2.—Incidence of diarrhea in 263 cases of acute bacillary dysentery and incidence of other symptoms in 237 cases of the disease.

LABORATORY FINDINGS

The total white blood cell count was recorded in 224 cases (chart 3). It varied rather widely, with extremes of 2,000 and 58,000, but in 90 per cent of cases it was between 4,000 and 13,000. The average figure was 8,760. The differential count was recorded in 217 cases (chart 4). It also varied widely. The number of neutrophilic granulocytes varied from 10 to 95 per cent of the total, but in 72 per cent of all cases it was between 60 and 85 per cent. The average was 67 per cent. The number of lymphocytes varied correspondingly, with an average of 33 per cent. Eosinophils were present in normal number, with an average of 0.9 per cent. The erythrocyte counts and hemoglobin determinations were not remarkable in any way and did not indicate the presence of significant dehydration. From these figures it can be seen that the blood count is not of much value in making a diagnosis of acute bacillary dysentery, as high, low or normal values may be present.

In 11 cases blood cultures were made; all were negative for the dysentery bacillus. It is generally agreed that blood cultures are of no value in diagnosis and that bacteremia is present only rarely.

In only 5 cases were agglutination tests performed. This number was so small that further study was not made and no conclusions can be drawn. Such tests are not of much value in diagnosis of the acute

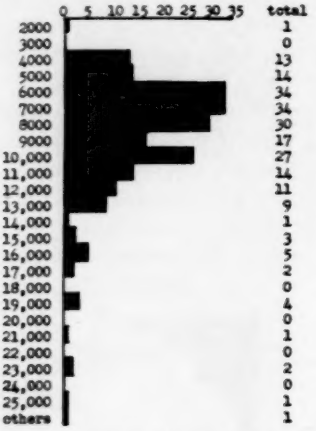

Chart 3.—White blood cell counts in 224 cases of acute bacillary dysentery.

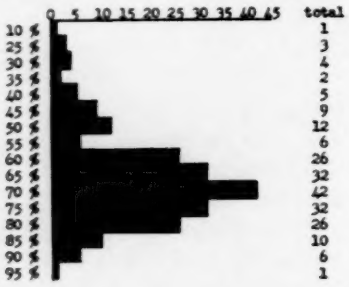

Chart 4.—Percentages of neutrophilic granulocytes in 217 cases of acute bacillary dysentery.

disease, since agglutinins do not appear in the blood stream until relatively late in the course of illness.

The results of gross and microscopic examination of the stools are given in table 4. The number of patients with blood, pus or mucus in the stools was not as high as might be expected. A possible explanation for this is the likelihood that many patients with a more benign form

of the disease may have entered the hospital too late in the course of illness for these substances to be found in a routine examination.

In 8 cases samples of urine were cultured; all were negative for the dysentery bacillus. In 3 cases, because of the presence of convulsions, spinal puncture was performed and the spinal fluid was cultured; all cultures were negative for the dysentery bacillus.

Wide differences of opinion exist regarding classification of the various dysentery bacilli, and there are several methods in use today. In this hospital the dysentery bacilli are classified as belonging to one of three groups, as follows:

Group 1: Non-mannitol-fermenting bacilli. Bacillus dysenteriae Shiga is the most important member of this group and is the only

TABLE 4.—*Results of Examination of Stools* *

Substance	No. of Cases	Percentage
Pus	159	79.5
Blood	131	65.5
Mucus	107	53.5

* One hundred and ninety-eight patients were examined.

TABLE 5.—*Incidence of Strains of Dysentery Bacilli*

Strain	No. of Cases	Percentage
Group 1	0	
Group 2	240	91.2
Group 3	17	6.5
Atypical	4	1.5
Mixed	2	0.8

dysentery bacillus known to produce an exotoxin. It possesses a distinctive type antigen.

Group 2: Mannitol-fermenting bacilli which do not ferment lactose and saccharose. Bacillus dysenteriae Flexner is the most generally known member of this group, but many other allied but slightly different strains have been described by Hiss, Russell, Strong, Musgrave and others.

Group 3: Mannitol-fermenting bacilli which are late fermenters of lactose and saccharose. The only known pathogenic member of this group is Bacillus dysenteriae Sonne.

Clinically bacillary dysentery may be divided into two types: (*a*) that due to infection with the Shiga bacillus, usually associated with a severe, toxic course and a high mortality, and (*b*) that due to any of the other strains, which characteristically induce a milder illness, with a much lower mortality.

The organisms recovered in the 263 cases are shown in table 5. It is of interest that no cases of infection with the Shiga bacillus were

encountered. Conner and Bates studied 66 cases of bacillary dysentery occurring in this hospital during the five year period from 1919 to 1923 and found that in 4 of the 66 infection was due to the Shiga bacillus.[4]

SIGMOIDOSCOPIC FINDINGS

One hundred and thirty-three, or 51 per cent, of all patients were examined by means of the sigmoidoscope on one or more occasions during their stay in the hospital. The reports submitted by the examiner are summarized in table 6. The percentage of positive findings was somewhat low. In many cases, however, the patient was examined only once, and in some cases this was done relatively late in the course of the disease. In a few instances the report simply read "characteristic of bacillary dysentery." Ulcers were observed in only 53 per cent of the patients examined.

TABLE 6.—*Results of Sigmoidoscopic Examination* *

Condition	No. of Cases	Percentage
Hyperemia	89	66.9
Ulcer	77	52.6
Edema	31	23.3

* One hundred and thirty-three examinations were made.

TREATMENT

Symptomatic measures were employed in all cases. This usually consisted of rest in bed, the administration of fluids and a simple liquid or low residue diet. In most cases an initial saline purge was given. Bismuth salts and camphorated tincture of opium were given almost routinely. In many cases scraped apple or pectin was given in varying quantity. In those cases in which sigmoidoscopic examination revealed a badly ulcerated rectum or sigmoid, local irrigation with a solution of tannic acid, silver nitrate or potassium permanganate was employed. The clinical evaluation of these many therapeutic measures does not lend itself well to statistical study. This is due to the multiplicity of measures employed, the lack of detailed comment on the progress of the patient and the impossibility of selecting a controlled series for the study of any one measure. No conclusions can be drawn.

Beginning in 1935, serum in the form of a polyvalent antidysentery preparation was employed. After that year such treatment was given

4. Conner, R. C., and Bates, L. B.: A Clinical and Bacteriological Analysis of the Cases of Bacillary Dysentery in Ancon Hospital During 1919 to 1923, Internat. Clin. **4**:34-47 (Dec.) 1924.

in 71.8 per cent of all cases, or 52 per cent of all cases in the series. It is unfortunate that the same serum preparation was not employed throughout this period. Instead, lots from several pharmaceutic houses were carried in stock at different times. In general it can be said that all consisted of immune serums obtained from horses which had been inoculated with several of the more common dysentery bacilli. The dose employed varied with several factors, depending on the age of the patient, the severity of the disease and the duration of the disease before treatment was instituted. In general, however, the amount varied from a total of 80 to 200 cc., given in several divided doses of 20 to 40 cc. by the intramuscular and/or the intravenous route. The dose for children was scaled accordingly.

It can be seen that as regards mortality, average number of days in the hospital and average number of febrile days, the serum-treated patients compare unfavorably with those who were not so treated (chart 5). The actual significance of these figures must be questioned,

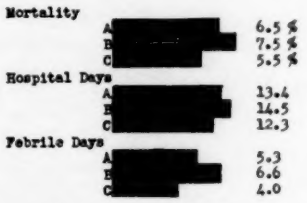

Chart 5.—Mortality, duration of stay in the hospital and duration of fever in the entire series of 263 cases (*A*), in 133 cases in which serum therapy was employed (*B*) and in 130 cases in which such therapy was not employed (*C*).

in view of the fact that, in general, the more seriously ill patients were selected for serum treatment whereas the patients with more benign disease were not so treated. Nevertheless, from the data collected, it cannot be said that the use of serum materially benefited the group of patients which were so treated.

A study of the available literature readily reveals that a wide difference of opinion exists regarding the efficacy of serum treatment of bacillary dysentery. Results of controlled studies are not available. The evidence regarding its use in cases of infection produced by the Flexner and Sonne organisms is particularly scanty. Striking results, with a reduction of mortality by 50 per cent and more in a group of cases produced by all dysentery bacilli, have been reported by Willmore and Savage.[5] Graham[6] stated that the mortality was reduced

5. Willmore, J. G., and Savage, H.: The Diagnosis and Treatment of Epidemic Bacillary Dysentery, Brit. M. J. **2**:1283-1286, 1913.

6. Graham, D.: The Diagnosis and Treatment of Bacillary Dysentery Occurring in the British Salonika Force, Lancet **1**:51-55 (Jan. 12) 1918.

to 1 per cent in 200 cases in which treatment was with serum. Acton and Knowles,[7] however, stated that the use of serum is of value only in infections caused by the Shiga bacillus. The most striking effect of serum therapy is its antitoxic effect in highly toxic patients, the majority of whom are infected with the Shiga bacillus. Cases of infection by this bacillus are not included in this study (table 5). Patients whose disease was of average severity or mild frequently respond with definite improvement, but since they are likely to improve in any case, the possibility of serum sickness and the expense of the serum must be considered before such therapy is attempted for them.

FEVER

In classifying these cases according to the height of fever, the predominant range of temperature was recorded, rather than the highest point reached. Eleven per cent of the patients were afebrile throughout their entire stay in the hospital. Conner and Bates [4] reported that

TABLE 7.—*Incidence of Fever*

Degree of Fever	Temperature, F.	No. of Cases	Percentage
None	.....	26	11.0
Low	98.6 to 100	51	21.7
Moderate	100 to 102	83	35.3
High	102 and over	75	31.9

nearly 10 per cent of their patients were afebrile. It can be seen that the patients with fever were distributed rather evenly through the low, moderate and high ranges (table 7).

DURATION OF HOSPITALIZATION

The number of hospital days for the entire series varied from three to thirty-seven, averaging thirteen and four-tenths. The number of febrile days varied from none to thirty-four, averaging five and three-tenths. It is evident that the disease, as it occurs on the Isthmus of Panama, is not incapacitating as regards length of stay in the hospital.

MORTALITY

Available statistics indicate that the mortality varies greatly in different localities and epidemics. The recorded figures run from 1 per cent to as high as 50 per cent. In the five year survey of cases occurring in this hospital during the years 1919 to 1923, previously referred to, the mortality rate was 28 per cent.[4] In the present series

7. Acton, H. W., and Knowles, R.: On the Dysenteries of India, Calcutta, Spink & Co., 1923.

of 263 cases there were 17 deaths, a mortality rate of 6.5 per cent. The mortality rate for the 237 cases in which there were no complications was 5.1 per cent (12 deaths). The number of days of hospitalization before death varied from one to nineteen and averaged six. In an attempt to evaluate the various factors concerned, the cases were grouped as shown in table 8. There was no variation between the sexes. The mortality among Negro patients was almost twice that among white patients. Age was of great importance. The greatest mortality was among the aged (40 per cent), with that among children occupying an intermediate position and with an exceedingly low death rate among all patients between the ages of 6 and 60 (2.4 per cent). The death rate among patients infected with the Sonne bacillus was

TABLE 8.—*Mortality*

	Percentage
Entire series	6.5
Sex	
Male	6.5
Female	6.5
Color	
Negro	8.1
White	4.5
Nationality	
British West Indian	10.9
Panamanian	8.0
American	2.2
Age, Years	
Under 1	20.0
1 to 5	15.5
6 to 60	2.4
Over 60	40.0
Dysentery bacilli	
Group two	6.3
Group three	11.8

almost twice that among patients infected with organisms belonging to the Flexner group.

EPIDEMIOLOGY

During the period studied there was only one true epidemic occurring in the Canal Zone. This was in February 1930, when over a period of thirteen days, 33 patients with acute bacillary dysentery were admitted to the hospital from the United States army posts of Corozal and Davis. In all cases infection was due to organisms belonging to the Flexner group and was relatively mild. There were no deaths. The cause of this epidemic was not determined.

It is likely, however, that infection in this epidemic, as well as in many isolated cases included in this study, was due to carriers. In a recent survey of all food handlers employed in the United States army posts of the Canal Zone, a total of 2,206 persons were subjected to

routine stool cultures. Of these, 5 were found to be carriers of dysentery bacilli. Two harbored bacilli of the Flexner type, while 3 carried bacilli of the Sonne type.

PATHOLOGY

Autopsy was performed in 13 (70 per cent) of the 17 fatal cases among the 263 cases in this series. One case was discarded because of the presence of pulmonary and intestinal tuberculosis. An additional 19 cases occurring before 1930 were added from the files. Thus a total of 31 autopsy protocols were studied.

Gross pathologic changes were recognized in 97 per cent of all cases, and the small intestine was involved almost as commonly as was the colon (table 9). The characteristic changes are confined to the colon and the lower portion of the ileum. The earliest change consists of enlarged lymph follicles standing out like grains of sand on a reddened

TABLE 9.—*Distribution of Gross Pathologic Lesions*

	No. of Cases	Percentage
Number of autopsies	31	
Gross changes recognized	30	97
Large intestine	29	93
Entire colon	24	
Cecum only	3	
Scattered	2	
Rectum only	1	
Small intestine	22	71
Distance from ileocecal valve	10 to 200 cm.	
Average		70 cm.

background of inflamed mucosa. Within a short time the centers of the follicles become necrotic, so that multiple miliary ulcers are produced. This necrosis is progressive, so that widespread, discrete and confluent ulceration is seen. The ulceration is not deep as a rule, and only rarely is the muscularis involved (4.5 per cent). The mucosa and submucosa, however, are necrotic over large areas, and in many cases (45 per cent) a well developed pseudodiptheritic membrane is formed.

Microscopic sections of the intestine show a heavy infiltration with neutrophilic granulocytes, lymphocytes and macrophages. This commonly involves the submucosa and muscularis and may extend to the serosa (14 per cent). As the process continues the coagulation necrosis becomes less marked, and small round cell infiltration is more prominent. Still later, granulation tissue may be observed growing up from the base of the ulcerated areas. These heal by extension of neighboring epithelium.

SUMMARY AND CONCLUSIONS

Data compiled from a study of 263 consecutive cases of acute bacillary dysentery have been presented.

The disease occurred among persons of each sex and of every age, color and nationality, but it predominated in Negroes (57 per cent), males (65 per cent) and patients aged 1 to 5 (17 per cent) and 21 to 25 (15 per cent).

Diarrhea, abdominal cramps, gross blood in the stool and fever were the most common symptoms, in the order given.

The total white blood cell and differential counts varied widely. The average for each was well within normal limits.

The organisms recovered from the stools consisted entirely of members of the Flexner (91 per cent) and the Sonne (6.5 per cent) group. No cases of infection with the Shiga bacillus were encountered.

Polyvalent serum therapy was employed in 52 per cent of all cases but was not adequately controlled in any sense. A study of the serum-treated, as compared with the non-serum-treated, patients failed to reveal evidence indicating that such therapy had been of value.

The mortality for the entire series was 6.5 per cent. It was greatest among the aged (40 per cent), with that among children occupying an intermediate position (15 to 20 per cent) and with an exceedingly low rate among all patients aged 6 to 60 (2.4 per cent). The mortality in cases of infection caused by the Sonne bacillus (11.8 per cent) was greater than in cases of infection caused by the Flexner bacillus (6.3 per cent).

The characteristic pathologic picture, as determined by a study of 31 autopsy protocols, has been described.

CHEMOTHERAPY AND CHEMOSEROTHERAPY OF STAPHYLOCOCCIC INFECTIONS

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AND
HERMAN BROWN, B.Sc.
WITH THE TECHNICAL ASSISTANCE OF ANNA M. RULE
PHILADELPHIA

Until the discovery of sulfanilamide and its derivatives chemotherapy had little of proved value to offer in the treatment of staphylococcic infections. It is true that recoveries from staphylococcic septicemia in human beings have been ascribed to the intravenous administration of gentian violet, neutral acriflavine and various mercurial and other compounds, but none of these substances has materially reduced its high mortality and none has proved of value in the treatment of experimental infections of mice and rabbits under well controlled conditions.

Domagk [1] found the original prontosil (the hydrochloride of 4-sulfamido-2′-4′-diaminoazobenzene) somewhat effective in the treatment of experimental staphylococcic infections of mice and later reported [2] that better results were observed with uliron (a brand of dimethyldisulfanilamide; paraaminobenzenesulfonylparaaminobenzenedimethylsulfonamide). As shown in table 1, various investigators have found sulfanilamide, sulfapyridine (2-[paraaminobenzenesulfonamido]-pyridine) and, more recently, thiazole derivatives of sulfanilamide effective in varying degrees in the treatment of these experimental infections. Sulfapyridine has been found somewhat more effective than sulfanilamide, while sulfathiazole (2-[paraaminobenzenesulfonamido]-thiazole) has proved more effective than sulfapyridine. The first published report on the therapeutic use of the original prontosil was that of Foerster,[3] who treated a child with staphylococcic septicemia; the child recovered. Since

From the Research Institute of Cutaneous Medicine.

1. Domagk, G.: Ein Beitrag zur Chemotherapie der bakteriellen Infektionen, Deutsche med. Wchnschr. **61**:250, 1935.

2. Domagk, G.: Weitere Untersuchungen über die chemotherapeutische Wirkung sulfonamidhaltiger Verbindungen bei bakteriellen Infektionen, Klin. Wchnschr. **16**:1412, 1937.

3. Foerster: Sepsis im Anschluss an ausgedehnte Periportis: Heilung durch Streptozon, Zentralbl. f. Haut- u. Geschlechtskr. **45**:549, 1933.

then it is astonishing that so few clinical results have been published,[4] but we surmise that this is not because the prontosils, sulfanilamide and sulfapyridine have failed to be employed but because the results have been poor and failures are so seldom reported.

It is true that sulfathiazole has proved more effective in the treatment both of local infections and of septicemia caused by staphylococci, with a material reduction in the mortality of the latter, but the chemotherapy of severe staphylococcic infections is by no means a solved problem, and

TABLE 1.—*Previous Reports of Experimental Infections with Staph. Aureus Induced in Mice and Treated with Sulfanilamide or a Derivative*

Author	Compound	Animals Treated		Controls	
		No.	Survival, %	No.	Survival, %
Buttle, G. A. H.: Proc. Roy. Soc. Med. **31**: 154, 1937	Sulfanilamide	30	50	10	0
Mellon, R. R.; Shinn, L. E., and McBroom, J.: Proc. Soc. Exper. Biol. & Med. **37**: 563, 1937	Sulfanilamide	21	38	21	14
Feinstone, W. H., and others: Bull Johns Hopkins Hosp. **62**: 565, 1938	Sulfanilamide	50	34	50	10
Whitby, L.: Lancet **2**: 1095, 1938.....	Sulfapyridine	40	15	18	16
	Sulfapyridine	80	7.5	..	..
Bliss, E. A., and Long, P. H.: Proc. Soc. Exper. Biol. & Med. **40**: 32, 1939	Sulfanilamide	50	8	20	0
	Sulfapyridine	49	33	..	..
Barlow, O. W., and Homburger, E.: Proc. Soc. Exper. Biol. & Med. **42**: 792, 1939	Sulfathiazole	20	70	20	0
	Sulfamethylthiazole	20	90	..	..
	Sulfaphenylthiazole *	20	45	..	..
	Sulfapyridine	20	30	..	..
Rake and McKee [5].....	Sulfapyridine	150	12	150	4.3
	Sulfathiazole	150	63.3	..	..
	Sulfamethylthiazole	150	52	..	..

* 2-(paraaminobenzenesulfonamido)-4-phenylthiazole.

none of the drugs of the sulfanilamide group has proved as effective as some of them are known to be in the treatment of hemolytic streptococcic infections.

There is therefore an urgent need for further efforts in the chemotherapy of staphylococcic septicemia. And this is particularly true since both mice and human beings may recover from the septicemia only later to succumb to pyemic abscesses in the kidneys or other organs. In other

4. Kolmer, J. A.: Progress in Chemotherapy of Bacterial and Other Diseases with Special Reference to Prontosils, Sulfanilamide and Sulfapyridine, Arch. Int. Med. **65**:671 (April) 1940.

words, sulfanilamide or one of its derivatives may effectively remove staphylococci from the blood but fail to disinfect the fixed tissues. This has also been shown to occur in experimental infections of mice, so that survival of animals for two to four weeks may not really indicate cure or complete recovery. For example, Rake and McKee [5] found that among mice surviving for thirty-one days after inoculation with Staphylococcus aureus, cultures of organs were positive for the organism in 14 per cent of those animals treated with sulfathiazole, in 18 per cent of those treated with sulfapyridine, in 25 per cent of those treated with sulfamethylthiazole (2-[paraaminobenzenesulfonamido]-4-methylthiazole) and in 12.5 per cent of the untreated controls. One of the purposes of the present investigation was to study this angle of the problem, and, as will be shortly shown, a large percentage of our surviving mice and rabbits had macroscopic or microscopic abscesses in the kidneys.

Under the conditions nothing of proved or helpful value can be neglected in the treatment of staphylococcic septicemia of human beings, in addition to therapy with drugs of the sulfanilamide group. Unfortunately, the older so-called polyvalent antistaphylococcus serums have generally proved ineffective. However, since numerous investigators have shown that therapy with a drug of the sulfanilamide group and serum combined has proved more effective in the treatment of experimental streptococcic,[6] meningococcic [7] and pneumococcic [8] infections of mice than therapy with either agent alone, we thought it advisable to determine the effectiveness of chemoserotherapy in the treatment of staphylococcic infections of mice, employing the newer staphylococcus antitoxin, especially since the last-named substance has given encouraging results in the treatment of experimental staphylococcic infections,[9] as well as in the treatment of septicemia in human beings.[10] In other

5. Rake, G., and McKee, C. M.: Action of Sulfathiazole and Sulfamethylthiazole on Staphylococcus Aureus, Proc. Soc. Exper. Biol. & Med. **43**:561, 1940.

6. Loewenthal, H.: Combined Serum and Sulfanilamide in the Treatment of Streptococcal Infections in Mice, Lancet **1**:197, 1939.

7. Branham, S. E., and Rosenthal, S. M.: Studies in Chemotherapy: Sulfanilamide, Serum, and Combined Drug and Serum Therapy in Experimental Meningococcus and Pneumococcus Infection in Mice, Pub. Health Rep. **52**:685, 1937.

8. MacLeod, C. M.: Chemotherapy of Pneumococcic Pneumonia, J. A. M. A. **113**:1405 (Oct. 7) 1939. Branham and Rosenthal.[7]

9. Burnet, F. M.: The Exotoxins of Staphylococcus Pyogenes Aureus, J. Path. & Bact. **32**:717, 1929. Parish, H. J.; O'Meara, R. A. J., and Clark, W. H. M.: The Clinical Investigation of Staphylococcal Toxin, Toxoid and Antitoxin, Lancet **1**:1054, 1934.

10. Panton, P. W.; Valentine, F. C. O., and Dix, V. W.: Staphylococcal Infection and Antitoxin Treatment, Lancet **2**:1180, 1931. Dolman, C. E.: Staphylococcus Antitoxic Serum in Treatment of Acute Staphylococcic Infections and Toxemia, Canada. M. A. J. **30**:601, 1934; **31**:1 and 130, 1934. Sutherland, R. T.: Staphylococcus Septicemia, Arch. Int. Med. **66**:1 (July) 1940.

words, there appears to be an important synergistic action between drugs of the sulfanilamide group and potent immune serums, and the results of our investigation reported here indicate that this action may be evinced in severe experimental staphylococcic infections.

MATERIAL AND METHOD

White mice were inoculated intravenously with 0.05 cc. of a highly virulent twenty-four hour broth culture of Staph. aureus. Of 120 untreated control animals, 18, or 15 per cent, survived twenty-one days. The kidneys of all treated and untreated mice, including those surviving this period, were examined macroscopically and microscopically for abscesses and the results recorded in tables 2 and 3.

Rabbits were given 0.1 cc. of eighteen hour broth cultures of the same strain intravenously once a day for eight days in succession in an effort to maintain a prolonged period of septicemia analogous to staphylococcic septicemia in human beings. Blood cultures made on the third, seventh, tenth and eighteenth days were positive for Staph. aureus in the majority of cases, and cultures made of the hearts of 15 animals immediately after death were all positive. The liver, kidneys, heart, etc., of all rabbits dying during treatment and those of all survivors were examined macroscopically and microscopically for abscesses; in table 4, 4 plus indicates the presence of numerous large and widely distributed abscesses, while 2 plus indicates the presence of microscopic abscesses only in the kidneys.

The shaved abdominal skin of additional rabbits was inoculated with 0.8 cc. of twenty-four hour broth culture of the staphylococcus. This dose was divided into four injections of 0.2 cc. each in a small area at the midline. Large lesions developed in about twenty-four hours, characterized by severe edema and hemorrhagic necrosis and similar to those produced by Kolmer and Rule[11] with hemolytic streptococcus and to those used by Kolmer, Brown, Rule and Werner[12] in a study of the therapeutic activity of sulfanilamide. In table 5, 4 indicates the presence of these large spreading lesions, due in most part to the presence of necrotizing toxin, while 3, 2 and 1 indicate lesions of progressively less severity. Cultures were made of material from the lesions, three, five, ten, fourteen and twenty-one days after inoculation and the results summarized in the table.

Azosulfamide (disodium of 4-sulfamidophenyl-2′-azo-7′-acetylamino-1′-hydroxynaphthalene-3′,6′-disulfonate), sulfanilamide, sulfapyridine, sulfathiazole and the following new compounds[13] were employed in treatment:

Aldanil: sodium formaldehyde sulfoxylate derivative of sulfanilamide

No. 2344: diaminodiphenylsulfone

No. 2439: p-cinnamalamino-p′-amidodiphenylsulfone[14]

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11. Kolmer, J. A., and Rule, A. M.: Experimental Streptococcus Infections in Rabbits for Therapeutic Investigations, J. Lab. & Clin. Med. **22**:1097, 1939.

12. Kolmer, J. A.; Brown, H.; Rule, A. M., and Werner, M. F.: Toxicity, Therapeutic Activity, and Mode of Action of Sulfanilamide in Experimental Streptococcus Infection of Rabbits, J. Lab. & Clin. Med. **24**:164, 1938.

13. These compounds were synthesized by Dr. George W. Raiziss, of the Dermatological Research Laboratories of the Abbott Laboratories, Inc., and supplied by him.

14. Raiziss, G. W.; Kolmer, J. A., and Rule, A. M.: Sulfone Compounds in the Treatment of Experimental Pneumococcal Infections, J. Infect. Dis. **66**:138, 1940.

No. 2879: sulfathiazoline (sulfhydrothiazole; 2-sulfanilyl-3,5-dihydrothiazole)[15]

No. 2898: tetramethyldecamethylenediamine-bis (4-chloracetamidophenyl)-sulfide polymeric quaternary salt

No. 2946: 2-(paraaminobenzenesulfonamido)-pyrimidine

No. 2949: acetaldehyde bisulfite derivative of sulfanilamide

No. 2955: sulfadimethylpyrimidine

All of the compounds were administered to the mice orally in doses of 0.1 Gm. per kilogram of weight. The drug was first given four hours after inoculation and every twelve hours thereafter for a total of ten doses, equivalent to 0.2 Gm. per kilogram of weight daily. Four additional doses of 0.1 Gm. were then administered daily for four days in succession. In the case of surviving mice the total dose of each compound was therefore 2.4 Gm. per kilogram of weight over a period of fourteen to fifteen days.

Additional mice were treated in the same manner with azosulfamide, sulfanilamide, sulfapyridine and aldanil except that globulin-modified staphylococcus antitoxin[16] was given intra-abdominally in doses of 0.1 cc. (80 units) per mouse. The antitoxin was first injected four hours after inoculation and every twelve hours thereafter for a total of ten doses, followed by four additional doses at daily intervals.

In the treatment of the rabbits the compounds were administered orally or intramuscularly in doses of 0.1 Gm. per kilogram of weight, the first being given three days after inoculation in order to permit the septicemia to become well established. Subsequent doses were given every twelve hours for eight days, equivalent to 0.2 Gm. per kilogram of weight daily. In the treatment of intradermal lesions the compounds were given orally, subcutaneously or intravenously in doses of 0.1 Gm. per kilogram of body weight every twelve hours for five to twelve doses, with the first dose administered four hours after inoculation.

RESULTS

Chemotherapy of Staphylococcic Infections in Mice.—The results observed with twelve compounds in the treatment of 316 mice are summarized in table 2. It will be observed that 14, or 15.5 per cent, of 90 additional untreated mice survived the period of observation of twenty-one days but all of these, as well as all mice which died, showed macroscopic or microscopic abscesses in the kidneys. The amount of culture employed therefore was about a single minimal lethal dose and thereby more favorable to the action of the compounds than an overwhelming infection would have been.

It will be observed that best results, from the standpoint both of survival and of escape from pyemic abscesses of the kidneys, were

15. Raiziss, G. W., and Clemence, L. W.: 2-Sulfanilyl-aminothiazoline, J. Am. Chem. Soc. **63**:3124, 1941. Raiziss, G. W.; Severac, M., and Moetsch, J. C.: Chemotherapeutic Studies of 2-Sulfanilyl 3-5 Dihydrothiazole (Sulfathiazoline), to be published.

16. The antitoxin was supplied by Lederle Laboratories, Inc.

observed with sulfathiazole, sulfathiazoline and sulfapyridine, especially sulfathiazole and sulfathiazoline, which appear to be equal in therapeutic effectiveness, since the slight differences observed were easily within the range of experimental error. Next best results were observed with sulfadimethylpyrimidine (no. 2955), followed in order by the acetaldehyde bisulfite derivative of sulfanilamide (no. 2949), 2-(paraaminobenzenesulfonamido)-pyrimidine (no. 2946), sulfanilamide, aldanil, azosulfamide and tetramethyldecamethylenediamine-bis (4-chloracetamidophenyl)-sulfide polymeric quaternary salt (no. 2898), while diaminodiphenylsulfone

TABLE 2.—*Results of Treatment of Mice Inoculated Intravenously with Staph. Aureus* *

Compound	No. of Doses †	No. of Mice	Survival, Days						Survival, %	Abscesses in Kidneys, %
			3	5	7	10	14	21		
Azosulfamide	14	24	18	16	16	14	8	8	33.3	91.7
Sulfanilamide	14	24	20	20	18	16	12	10	41.7	83.3
Sulfapyridine	14	52	50	47	40	38	31	27	51.9	50
Sulfathiazole	14	42	40	38	38	31	28	24	57.1	48
Sulfathiazoline	14	40	40	40	36	30	27	22	55	50
Aldanil	14	34	31	28	19	16	14	12	35.3	100
No. 2344 ‡	14	12	7	0	0	0	0	0	0	100
No. 2439 §	14	12	3	1	0	0	0	0	0	100
No. 2898 ‖	14	12	12	10	6	4	4	4	33.3	66.6
No. 2946 ¶	14	14	14	12	12	10	8	6	42.8	71.4
No. 2949 #	14	20	20	15	12	11	10	9	45	60
No. 2955 **	14	30	26	26	24	23	18	15	50	80.6
Controls	..	90	73	60	35	22	14	14	15.5	100

* The inoculum consisted of 0.05 cc. of a twenty-four hour broth culture.

† In doses of 0.1 Gm. per kilogram of weight the drugs were first given four hours after inoculation and were administered every twelve hours thereafter for a total of ten doses, followed by one dose daily for four successive days.

‡ Diaminodiphenylsulfone.

§ P-cinnamalamino-p'-amidodiphenylsulfone.

‖ Tetramethyldecamethylenediamine-bis (4-chloracetamidophenyl)-sulfide polymeric quaternary salt.

¶ 2-(paraaminobenzenesulfonamide)-pyrimidine.

Acetaldehyde bisulfite derivative of sulfanilamide.

** Sulfadimethylpyrimidine.

(no. 2344) and p-cinnamalamino-p'-amidodiphenylsulfone (no. 2439) were completely ineffective. It will also be noted that the incidence of abscesses in the kidneys showed a close relation to the therapeutic effectiveness of the compounds on the basis of percentage of survival.

Chemoserotherapy of Staphylococcic Infections in Mice.—The results observed in the treatment of 120 additional mice with six of the compounds alone and of 120 with the compounds combined with staphylococcus antitoxin are summarized in table 3. Of 30 untreated controls, 4, or 13.3 per cent, survived, although all 30 showed abscesses in the kidneys. Of 30 mice treated with the antitoxin alone, 10, or 33.3 per cent, survived and 66.6 per cent of the group showed abscesses in the

kidneys. The results have shown, therefore, that the staphylococcus antitoxin in the dose employed had definite therapeutic effects.

Sulfathiazole, sulfathiazoline and sulfapyridine alone again gave the best therapeutic results, followed in order by sulfanilamide, azosulfamide and aldanil. But it will be observed that combined treatment with one of the compounds and staphylococcus antitoxin gave better results from the standpoint both of percentage of survival and of incidence of abscesses in the kidneys than did therapy with any single compound. While staphylococcus antitoxin is administered to human beings primarily for

TABLE 3.—*Results of Chemotherapy of Mice Inoculated Intravenously with Staph. Aureus* *

Compound	Treatment: Doses Cpd.†	Treatment: Doses of Antitoxin‡	No. of Mice	Survival, Days: 3	5	7	10	14	21	Survival, %	Abscesses in Kidneys, %
Azosulfamide	14	0	20	16	12	12	10	8	8	40	90
Azosulfamide	14	14	20	16	16	15	14	14	14	70	70
Sulfanilamide	14	0	20	18	18	16	16	8	8	40	80
Sulfanilamide	14	14	20	20	20	18	14	12	10	50	60
Sulfapyridine	14	0	20	18	18	16	14	12	10	50	60
Sulfapyridine	14	14	20	19	18	18	16	14	12	60	50
Sulfathiazole	14	0	20	20	20	18	16	13	12	60	50
Sulfathiazole	14	14	20	20	20	20	18	15	14	70	45
Sulfathiazoline	14	0	20	20	20	20	16	11	11	55	60
Sulfathiazoline	14	14	20	20	20	19	17	15	13	65	50
Aldanil	14	0	20	14	12	8	4	4	4	20	100
Aldanil	14	14	20	18	18	16	10	10	10	50	60
Antitoxin-treated controls	0	14	30	24	18	15	12	10	10	33.3	66.6
Untreated controls	0	0	30	24	16	12	7	4	4	13.3	100

* The initial inoculum consisted of 0.05 cc. of a twenty-four hour broth culture.

† In doses of 0.1 Gm. per kilogram of weight the drugs were first given four hours after inoculation and were administered every twelve hours thereafter for a total of ten doses, followed by one dose daily for four successive days.

‡ Staphylococcus antitoxin was administered intra-abdominally in doses of 0.1 cc. per mouse. It was first given four hours after inoculation and was administered every twelve hours thereafter for a total of ten doses, followed by one dose daily for four successive days.

the relief of toxemia, it appears from the results of this experimental study to have a synergistic effect, with a distinct improvement in the therapeutic effectiveness of the drugs of the sulfanilamide group. For this reason we believe it is advisable to employ staphylococcus antitoxin in large doses, and preferably by intravenous administration, along with such drugs in the treatment of staphylococcic septicemia in human beings.

Chemotherapy of Staphylococcic Infections in Rabbits.—The first experiment consisted in the daily intravenous inoculation of 60 rabbits with 0.1 cc. of an eighteen hour broth culture of Staph. aureus for eight days, which produced septicemia in the majority of the animals. Treatment began on the third day, at which time blood cultures positive for the staphylococcus were obtained, a dose being given every twelve hours

for a total of sixteen doses over a period of eight days. In other words, the compounds were administered twice daily for five days along with a daily inoculation of staphylococci and were continued for three days after inoculation had been stopped.

As shown in table 4, 2 of the 6 untreated controls survived eighteen days, but these, as well as the 4 animals which died, showed numerous abscesses in the kidneys and other organs.

On the seventh day of treatment the blood of rabbits given sulfanilamide contained 1.2 to 6.8 mg. of free sulfanilamide per hundred cubic centimeters; that of those given azosulfamide orally contained 2.2 to

TABLE 4.—*Treatment of Rabbits Inoculated Intravenously with Staph. Aureus* *

Compound	Administration † Dose, Gm. per Kg.	Route	No. of Rabbits	Survival, Days 3	5	7	10	14	18	Abscesses Evident at Necropsy ‡
Sulfanilamide	0.1	Oral	6	6	6	6	5	3	3	++++
Azosulfamide	0.1	Oral	6	6	5	4	3	3	3	++++
Azosulfamide	0.1	Intramuscular	6	6	5	5	4	3	3	++
Sulfapyridine	0.1	Oral	6	6	6	6	6	4	4	++
Sulfathiazole	0.1	Oral	6	6	6	6	6	5	5	++
Aldanil	0.1	Oral	6	6	6	6	5	4	2	++
Aldanil	0.1	Intramuscular	6	6	6	6	5	5	2	++
No. 2344 §	0.1	Oral	6	6	6	5	4	2	2	++++
No. 2439 ‖	0.1	Oral	6	6	5	5	3	2	2	++++
Controls	...	.....	6	6	5	3	3	2	2	++++

* The inoculum consisted of 0.1 cc. of an eighteen hour broth culture daily for eight doses.

† The first dose was given on the third day, and the drug was administered every twelve hours for eight days.

‡ ++, microscopic abscesses only in the kidneys; ++++, large and numerous abscesses in the kidneys, heart muscle, etc.

§ Diaminodiphenylsulfone.

‖ P-cinnamalamino-p'-amidodiphenylsulfone.

3.2 mg. of drug, while that of animals given the latter compound intramuscularly contained 0.8 to 2.8 mg. The level of free drug in the blood of rabbits given sulfapyridine was 0.7 to 4.5 mg., per hundred cubic centimeters, while that in the blood of animals given sulfathiazole was 0.5 to 4.4 mg.

As shown in the table, best results were obtained with sulfathiazole and sulfapyridine; sulfathiazoline was not employed as it was not available at the time the experiment was conducted. It is possible that sulfanilamide and azosulfamide were slightly effective, but aldanil and compounds 2344 and 2439 had no demonstrable therapeutic effect.

The same seven compounds with the exception of sulfathiazole were used in the treatment of 27 rabbits inoculated intradermally with Staph. aureus in amounts of 0.1 Gm. per kilogram of weight. The first dose

was given four hours after intradermal inoculation, and the drug was administered every twelve hours thereafter for a total of twelve doses, except in the case of 1 animal which received a total of five doses and

TABLE 5.—*Treatment of Rabbits Inoculated Intracutaneously with Staph. Aureus* *

	Administration †		Lesions,‡ Days							Cultures of Material from Lesions, Days				
Compound	Route	No. of Doses	1	2	3	5	7	14	21	3	5	10	14	21
Sulfanilamide	Oral	8	4	4	4	D	..	..	..	+	+	..	..	..
Sulfanilamide	Oral	12	4	3	3	2	2	1	—	+	+	+	—	—
Sulfanilamide	Oral	12	4	4	3	3	2	1	—	+	+	+	—	—
Sulfanilamide	Subcutaneous	12	4	4	4	4	2	—	—	+	+	+	+	—
Sulfanilamide	Subcutaneous	12	4	4	4	4	3	1	—	+	+	+	+	—
Sulfanilamide	Subcutaneous	12	4	4	4	3	3	—	—	+	+	+	+	—
Azosulfamide	Oral	12	4	3	2	2	2	2	—	+	+	+	+	—
Azosulfamide	Oral	12	4	3	3	2	2	2	—	+	+	+	—	—
Azosulfamide	Oral	12	4	4	3	2	1	—	—	+	+	+	—	—
Azosulfamide	Subcutaneous	12	4	4	4	3	3	—	—	+	+	+	—	—
Azosulfamide	Subcutaneous	12	4	4	4	3	2	—	—	+	+	+	—	—
Azosulfamide	Subcutaneous	12	4	4	3	3	2	1	—	+	+	+	—	—
Sulfapyridine	Oral	12	4	4	4	4	3	—	—	+	+	—	—	—
Sulfapyridine	Oral	12	4	4	4	4	3	2	—	+	+	+	+	—
Sulfapyridine	Oral	12	4	4	4	3	2	—	—	+	+	+	—	—
Sulfapyridine	Intravenous	5	4	4	D	..	..	..	..	+	..	..	..	..
Sulfapyridine	Intravenous	12	4	2	2	—	—	—	—	+	+	—	—	—
Sulfapyridine	Intravenous	12	4	4	2	2	—	—	—	+	+	—	—	—
Aldanil	Oral	12	4	4	3	2	2	2	—	+	+	—	—	—
Aldanil	Oral	12	4	4	4	3	2	2	1	+	+	—	—	—
Aldanil	Oral	12	4	4	3	2	2	2	—	+	+	—	—	—
No. 2344	Oral	12	4	4	4	3	2	2	1	+	+	+	+	—
No. 2344	Oral	12	4	4	4	3	1	D	..	+	+	..	..	..
No. 2344	Oral	12	4	4	4	3	2	2	1	+	+	+	+	—
No. 2439	Oral	12	4	4	4	3	2	2	1	+	+	+	+	—
No. 2439	Oral	12	4	4	4	3	3	3	2	+	+	+	+	—
No. 2439	Oral	12	4	4	4	3	2	2	2	+	+	+	+	—
Control	.....	..	4	4	4	4	3	3	1	+	+	+	+	+
Control	.....	..	4	4	4	4	3	1	—	+	+	+	—	—
Control	.....	..	4	4	4	3	3	2	1	+	+	+	—	—

* The inoculum consisted of 0.8 cc. of a twenty-four hour broth culture.

† In doses of 0.1 Gm. per kilogram of weight the drugs were first given four hours after inoculation and were administered every twelve hours thereafter.

‡ The following descriptive symbols are used: 4, large spreading lesions; 3, 2 and 1, local lesions of progressively less severity; —, absence of lesions, and D, death of animal.

1 which received eight doses. The routes of administration are shown in table 5.

Cultures were made of material from the lesions three, five, ten, fourteen and twenty-one days after inoculation, with the results shown in table 5. A determination of free sulfanilamide in the blood of the rabbits given this drug orally was made four hours after the fifth dose and showed 1.8 to 2.6 mg. per hundred cubic centimeters; similar levels

were observed in those rabbits given the compound subcutaneously. In the rabbits given azosulfamide orally the concentration of free drug was 0.5 to 0.8 mg. per hundred cubic centimeters, and in those given the drug by subcutaneous injection the level was 0.5 mg. In those given sulfapyridine orally the concentration was 0.5 to 1.0 mg. per hundred cubic centimeters, and in those receiving it intravenously the level was 0.8 mg.

All 3 untreated controls survived but showed severe local lesions, with cultures positive for the staphylococcus during the first seven days after inoculation. That 2 recovered spontaneously was indicated by cultures made from material taken from the lesions on the twenty-first day which remained negative for the causative organism.

Of the 27 treated animals, 3 died during the period of observation, with cultures of material taken from a lesion which were positive for the staphylococcus. Cultures of heart blood from these rabbits made immediately after death were positive for the staphylococcus.

It is difficult, however, to draw any deductions from the results except that sulfapyridine was apparently the most effective of the six compounds. Sulfathiazole and sulfathiazoline were not employed, as they were not available at the time the experiment was conducted. Aldanil and compounds 2344 and 2439 were ineffective, while sulfanilamide and azosulfamide yielded doubtful results.

SUMMARY

Sulfathiazole and sulfathiazoline have proved most effective in the treatment of experimental Staph. aureus infections in mice. Sulfapyridine ranked third and sulfadimethylpyrimidine fourth in therapeutic effectiveness.

The complete cure of mice, however, is not indicated by the survival rates, as varying percentages of mice surviving twenty-one days have shown the presence of pyemic abscesses in the kidneys. There is, however, a relation between the percentage of survival and the incidence of renal abscesses.

The simultaneous administration of staphylococcus antitoxin and drugs of the sulfanilamide group (chemoserotherapy) has given somewhat better therapeutic results than therapy with the latter agents alone in the treatment of mice inoculated with Staph. aureus.

Sulfathiazole and sulfapyridine also proved most effective of the compounds used in the treatment of staphylococcic septicemia in rabbits. Sulfathiazoline was not employed. All of the animals surviving eighteen days, however, showed the presence of pyemic abscesses in the kidneys or other organs, so that survival alone is not an index of complete cure.

Sulfapyridine was also somewhat effective in the treatment of local lesions produced by the intradermal inoculation of Staph. aureus. Sulfathiazole and sulfathiazoline were not employed.

Two sulfone compounds were ineffective in the treatment of experimental staphylococcic infections in mice and rabbits.

The results have indicated, therefore, that sulfathiazole, sulfathiazoline and sulfapyridine possess therapeutic activity against experimental staphylococcic infections but frequently fail to bring about complete recovery insofar as pyemic abscesses of the kidneys and other internal organs are concerned. Under the circumstances, it is concluded that the chemotherapy of staphylococcic infections is still unsatisfactory and certainly inferior to therapy with the drugs of the sulfanilamide group in hemolytic streptococcic and pneumococcic infections.

QUANTITATIVE STUDIES ON ANTITHROMBIN

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The failure of the circulating blood to clot spontaneously under physiologic conditions in vivo still remains a unique and obscure phenomenon, since all of the elements essential for immediate coagulation in vitro are apparently present. Howell, in his fundamental concept of blood coagulation, has consistently maintained in at least partial explanation of this enigma that substances acting as clotting inhibitors are normally present in blood plasma. With the identification of heparin by Howell and Holt [1] and the demonstration of the presence of proantithrombin, the nature and role of these anticoagulants in the blood began to be better understood. Evidence has been presented suggesting that the principal anticoagulant effect of heparin is the prevention of the conversion of prothrombin to thrombin. However, when added to plasma, heparin apparently activates the proantithrombin, resulting in a tremendous increase in antithrombic activity. Charles and Scott,[2] among others, have confirmed the presence of heparin in blood and isolated 185 units of crude heparin from 1 Kg. of ox plasma and a negligible amount from ox serum. Jerpes, Holmgren and Wilander [3] have recently suggested that the site of formation of heparin is in the mast cells of Ehrlich. Thus these cells, which are widespread throughout the body tissues, may constitute a "secretory organ" the function of which is to maintain the fluid character of the blood in vivo.

The normally existing anticoagulants, or clotting inhibitors, in blood serum and plasma, according to the present concepts, are antiprothrom-

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1. (*a*) Howell, W. H., and Holt, E.: Two New Factors in Blood Coagulation: Heparin and Pro-Antithrombin, Am. J. Physiol. **47**:328-341, 1918. (*b*) Howell, W. H.: The Purification of Heparin and Its Presence in Blood, ibid. **71**:553-562, 1925.

2. Charles, A. F., and Scott, D. A.: Studies in Heparin: II. Heparin in Various Tissues, J. Biol. Chem. **102**:431-435, 1933.

3. Jerpes, E.: On Heparin: Its Chemical Nature and Properties, Acta med. Scandinav. **88**:427-433, 1936. Holmgren, H., and Wilander, O.: Beitrag zur Kenntnis der Chemie und Funktion der Ehrlichschen Mastzellen, Ztschr. f. mikr.-anat. Forsch. **42**:242-278, 1937. Wilander, O.: Studien über Heparin, Skandinav. Arch. f. Physiol. (supp. 15) **81**:1, 1938.

bin, antithromboplastin and antithrombin (fig. 1). Antiprothrombin prevents the conversion of prothrombin to thrombin. There is some doubt as to the exact nature of antiprothrombin, the accumulated evidence suggesting that the prevention of the conversion of prothrombin to thrombin is due to an antithromboplastic action. Brinkhous, Smith, Warner and Seegers [4] have shown that heparin requires an additional plasma factor before it can prevent the formation of thrombin and have postulated that the anticoagulant effect may be due to an antithromboplastic rather than an antiprothrombic effect. Ferguson [5] expressed a preference for the view that the antiprothrombic effect of heparin is to be interpreted not as an alteration in prothrombin itself but rather as a manifestation of antagonisms between heparin and thromboplastic factors. Howell [6] has stated on numerous occasions that thromboplastin will neutralize the effect of heparin and has postulated that thromboplastin combines with the antiprothrombin of the antiprothrombin-prothrombin combination, resulting in the release of free prothrombin. Sterner and

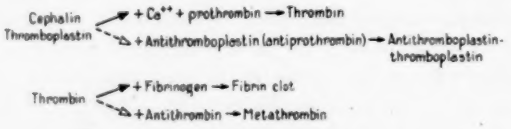

Fig. 1.—The sequence of events in blood coagulation, illustrating the possible fate of cephalin, thromboplastin and thrombin.

Medes [7] have shown that cysteine and other cystine derivatives have an antiprothrombic action.

The plasma of normal human subjects contains 300 units of prothrombin per cubic centimeter, 1 unit being defined as that amount which when converted to thrombin will clot 1 cc. of fibrinogen in fifteen seconds.[8] Thus plasma contains sufficient potential thrombin in the

4. Brinkhous, K. M.; Smith, H. P.; Warner, E. D., and Seegers, W. H.: The Inhibition of Blood Clotting: An Unidentified Substance Which Acts in Conjunction with Heparin to Prevent the Conversion of Prothrombin into Thrombin, Am. J. Physiol. **125**:683-687, 1939.

5. Ferguson, J. H.: Heparin and Plasma Albumin in Relation to Thromboplastic Action of Trypsin, Cephalin and Brain Extracts, Proc. Soc. Exper. Biol. & Med. **42**:33-37, 1939.

6. (*a*) Howell, W. H.: Theories of Blood Coagulation, Physiol. Rev. **15**:435-470, 1935. (*b*) Footnote 1.

7. Sterner, J. H., and Medes, C.: The Effect of Certain Sulfur Compounds on the Coagulation of Blood, Am. J. Physiol. **117**:92-101, 1936.

8. Smith, H. P.: Prothrombin and Thrombin, read at the meeting of the American Association for the Advancement of Science, Columbus, Ohio, Dec. 27, 1939.

form of its precursor prothrombin to clot 300 cc. of fibrinogen in fifteen seconds. When normal blood is allowed to clot the thrombin is almost entirely inactivated or neutralized in one or two hours. Brinkhous, Smith and Warner [9] have shown that hemorrhage may occur when the prothrombin level is below 35 per cent of normal, i. e., below 105 units per cubic centimeter. Persons with this degree of hypoprothrombinemia have sufficient potential thrombin in the form of the precursor prothrombin to clot 105 cc. of fibrinogen in fifteen seconds. The occurrence of hemorrhage is only partially explained on the basis of decreased levels and the convertibility of plasma prothrombin. From these observations it is quite evident that the thrombin must be inactivated or neutralized in such proportions that a hemorrhagic tendency develops. However, an analysis of the available methods for the quantitative determination of antithrombin would indicate that serum or plasma can inactivate only a fraction of a unit of thrombin in ten to fifteen minutes. Howell [6a] has stated that blood contains but a small amount of thrombin. Therefore it is obvious that either the antithrombic activity is much greater than measured by previous methods or the thrombin is neutralized or inactivated in some other manner.

Quantitative determinations of antithrombin in blood serum and plasma have not been entirely satisfactory up to the present time because of the lack of quantitative data relating to the various other essential elements in the blood-clotting mechanism. The various methods which, from time to time, have been suggested for the determination of antithrombin and of other anticoagulants have given only qualitative or crude quantitative estimations. Howell [10] and Gasser [11] observed the antithrombic activity of serum which had been heated to remove all traces of thrombin and fibrinogen. Thrombin was then added and after suitable periods of incubation the ability of the mixture to coagulate fibrinogen was determined. Mills and Kitzmiller [12] used a similar method in studying the antithrombin of blood serum from patients with typhoid fever; their data showed an increase. Quick [13] and Eagle, Johnston and

9. Brinkhous, K. M.; Smith, H. P., and Warner, E. D.: Prothrombin Deficiency and the Bleeding Tendency in Obstructive Jaundice and in Biliary Fistula, Am. J. M. Sc. **196**:50-57, 1938.

10. Howell, W. H.: The Condition of Blood in Hemophilia, Thrombosis and Purpura, Arch. Int. Med. **13**:76-95 (Jan.) 1914.

11. Gasser, W. H.: The Significance of Prothrombin and of Free and Combined Thrombin in Blood-Serum, Am. J. Physiol. **42**:378-394, 1916.

12. (*a*) Mills, C. A., and Kitzmiller, K. V.: Aid in Diagnosis of Typhoid Fever: New Laboratory Method, Arch. Int. Med. **38**:544-550 (Oct.) 1926. (*b*) Mills, C. A.: Antithrombin Test in Typhoid Fever: Improvement in Technic, ibid. **39**:618-620 (May) 1927.

13. Quick, A. J.: The Normal Antithrombin of the Blood and Its Relation to Heparin, Am. J. Physiol. **123**:712-719, 1938.

Ravdin [14] added various amounts of thrombin to plasma and observed the variations in coagulation time. These investigators used their methods for determining experimental increases in antithrombin and apparently never intended them to be used for observations on normal plasma. The method of Chargaff, Bancroft and Stanley-Brown,[15] in which an inhibitor unit is defined, is not well adapted to the study of antithrombin, as both the antiprothrombin and the antithrombin must be observed in one step. Heparin units, as defined by Howell,[16] cannot be readily converted into corresponding quantities of antithrombin per se.

With the recent development of a technic for the standardization and purification of thrombin by Warner, Brinkhous, Smith and Seegers,[17] it has become possible to devise an adequate method for the quantitative determination of antithrombin.[18] It must be obvious that any method for the determination of antithrombin involves the principle of the inactivation of thrombin. Before such a method could be devised, however, various quantitative observations were necessary on the inactivation of thrombin by potassium oxalate, blood plasma and serum. The disappearance of thrombin during the conversion of fibrinogen to fibrin was measured quantitatively. Observations were then made on the inactivation of thrombin by diluted and undiluted serum and plasma. With these data a dependable test has been devised for the quantitative determination of antithrombin. The normal levels of antithrombin were then determined in normal human subjects and in various laboratory and domestic animals.

METHOD

The prothrombin-free fibrinogen was prepared by the method of Warner, Brinkhous and Smith,[17b] all procedures being carried out at 5 C. and the materials stored at —35 C. The thrombin was prepared by the method of Seegers, Brinkhous, Smith

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14. Eagle, H.; Johnston, C. C., and Ravdin, I. S.: On the Prolonged Coagulation Time Subsequent to Anaphylactic Shock, Bull. Johns Hopkins Hosp. **60**:428-438, 1937.

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18. Wilson, S. J.: Quantitative Studies on Antithrombin, Proc. Soc. Exper. Biol. & Med. **43**:676-678, 1940.

and Warner and standardized by either the pipet or the dropper method. The standardization tests should be repeated just before any critical observations are made.

The following method was devised to determine accurately fractions of 1 unit of thrombin: Fifteen drops of physiologic solution of sodium chloride was added to each of 9 test tubes. A series of 9 dilutions containing 0.2 to 1.0 unit of thrombin in each drop was then prepared, and portions of a single drop were added in sequence to the tubes of saline solution. To each test tube was then added 5 drops of fibrinogen (a total of 21 drops), and the appearance of the fibrin strands was timed with a stopwatch. The following clotting times were observed:

Units of Thrombin	Clotting Time, Sec.
1.0	20
0.9	21.5
0.8	23.5
0.7	25.5
0.6	27.5
0.5	31
0.4	35.5
0.3	40.5
0.2	46.5

A curve to be used in the studies on quantitative antithrombic activity was then plotted. One unit of thrombin was used in the studies. If the figures for the thrombin are reversed the curve will indicate the amount of thrombin inactivated or neutralized.

QUANTITATIVE ANTITHROMBIC ACTIVITY OF SERUM AND PLASMA

Blood was obtained from a rabbit by heart puncture, and a portion was mixed with one seventh of its volume of a 1.85 per cent solution of potassium oxalate. The serum and plasma were removed after centrifugation. Portions of the serum and plasma were incubated at 63 C. for ten minutes and the precipitated fibrinogen of the plasma discarded. A part of the incubated plasma was placed in a cellulose membrane and dialyzed against cold distilled water until the potassium oxalate and other electrolytes were removed, this point being easily determined by the precipitation of the water-insoluble euglobulin fraction.

Dilutions of the plain serum, the incubated serum and the plain and the dialyzed incubated oxalated plasma of 1:65 were prepared with physiologic solution of sodium chloride and corrections made for dilution by the oxalate solution or increase in volume by dialysis. A series of test tubes was set up for each specimen with 15 drops of serum or plasma in each tube. One unit (1 drop) of thrombin was added to each tube and incubated at 28 C. for various intervals, after which 5 drops of fibrinogen was added. The clotting times were observed and the amount of thrombin inactivated was determined. There was but a negligible difference in the antithrombic activity in the various prepa-

rations, all variations being within the range of experimental error (fig. 2). Potassium oxalate will inactivate thrombin, this effect apparently being lost in dilutions as great as were used in these observations. The blood should not be mixed with greater than one seventh of its volume of a 1.85 per cent solution of potassium oxalate. This substance does not activate the proantithrombin of the plasma as does heparin. Dialysis with the cellulose membrane did not result in a decrease in the ability of the plasma to inactivate thrombin.

The antithrombic activity of undiluted serum and plasma was then determined (table 1). The following specimens were prepared from rabbit's blood: serum, serum incubated at 56 C. for ten minutes, oxalated plasma (2.8 mg. of potassium oxalate per cubic centimeter) incubated at 56 C. for ten minutes to precipitate and remove the fibrinogen and

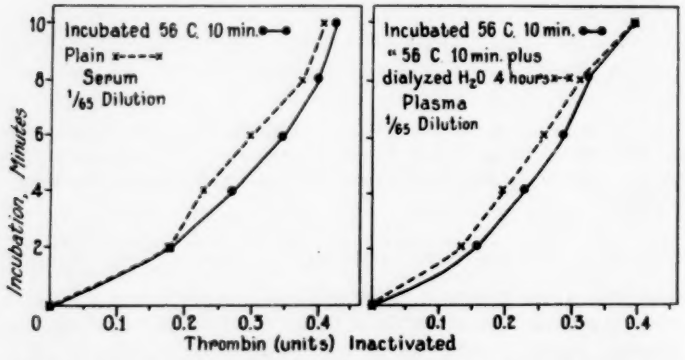

Fig. 2.—The quantitative antithrombic activity of saline dilutions of serum, incubated serum, incubated plasma and dialyzed incubated plasma in a physiologic solution of sodium chloride. The curves of activity are essentially the same.

TABLE 1.—*Inactivation of Thrombin by Undiluted Serum and Plasma (Rabbit)*

Specimen	Units of Thrombin Added per Cc.	Units of Thrombin Inactivated in 4 Minutes
Serum, plain	140	126
Serum, incubated 10 min. at 56 C.	140	124
Plasma, oxalated, fibrinogen removed by incubating for 10 min. at 56 C.	140	132
Plasma, oxalated, fibrinogen removed by adding 3 units of thrombin per cubic centimeter and removing fibrin clot	140	133

plasma from which the fibrinogen had been removed after having been converted to fibrin by the addition of 3 units of thrombin per cubic centimeter. To 1 cc. of each specimen was added 140 units of thrombin (0.1 cc.) and the amount calculated that was inactivated in four minutes'

incubation at 28 C. The amount of thrombin inactivated by the potassium oxalate was less than 0.1 unit; so no correction was necessary. Although plasma appeared to have a greater amount of antithrombic activity than serum, the difference was less than 6 per cent, a variation well within the present range of experimental error. Further detailed investigation is necessary to establish whether such relatively minor variations are constant and of any significance.

DISAPPEARANCE OF THROMBIN FROM SOLUTION DURING THE CONVERSION OF FIBRINOGEN TO FIBRIN

A prothrombin-free solution of fibrinogen was prepared, with a physiologic solution of sodium chloride used as the diluent. Oxalated

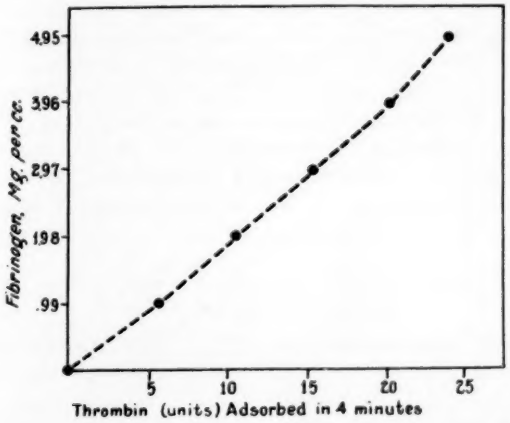

Fig. 3.—The adsorption of thrombin by various quantities of fibrinogen when converted to fibrin. Each milligram of fibrin adsorbed 5.1 units of thrombin.

saline solutions of fibrinogen were not used because of the thrombin-neutralizing power of potassium oxalate. The fibrinogen concentration was 4.95 mg. per cubic centimeter, as measured by the method of Greenberg and Mirolubova.[19]

Quantitative observations were then made on the amount of thrombin adsorbed by the conversion of varying amounts of fibrinogen to fibrin (fig. 3). A series of dilutions of various concentrations of fibrinogen was prepared. To 1 cc. of each dilution was added 35 units of thrombin, which resulted in the rapid and complete conversion of fibrinogen to

19. Greenberg, D. M., and Mirolubova, T. N.: Modifications in the Colorimetric Determinations of the Plasma Proteins by the Folin Phenol Reagent, J. Lab. & Clin. Med. **21**:431-435, 1936.

fibrin within a few seconds. The fibrin clot was removed from each tube after four minutes' incubation at 28 C. and the amount of thrombin accurately determined in the remaining liquid medium. From these data the quantity of thrombin which had disappeared from solution was calculated. When the curve was plotted the amount of thrombin utilized or adsorbed on the fibrin strands was shown to be in direct proportion to the concentration of the fibrinogen. Thus, the conversion of 1 mg. of fibrinogen to fibrin caused the disappearance of an average of 5.1 units of thrombin.

The question next to be decided was whether the fibrinogen when converted to fibrin in the presence of rather high concentrations of thrombin adsorbed its maximum amount of thrombin in four minutes or whether the adsorption process continued with longer periods of incubation. A series of 7 test tubes was prepared, each containing 1 cc. of fibrinogen (4.95 mg.). To each tube was added 55 units of thrombin. After various suitable periods of incubation, ranging from one to twenty-four minutes at 28 C., there was no variation in the amount of adsorbed thrombin calculated when the fibrin clot was removed. Apparently the saturation point of the adsorption of thrombin on the fibrin strands is quickly reached when the conversion of fibrinogen to fibrin occurs in the presence of excess amounts of thrombin.

ANTITHROMBIC ACTIVITY OF DILUTED AND UNDILUTED SERUM AND PLASMA

Rabbit plasma was incubated at 56 C. for ten minutes and the precipitated fibrinogen removed by centrifugation. Dilutions of the plasma of 1 : 10, 1 : 20, 1 : 30, 1 : 40, 1 : 50 and 1 : 60 were prepared with physiologic solution of sodium chloride, and the antithrombic activity of each was recorded for various periods of incubation at 28 C. After plotting the curves of each dilution, it was noted that there was a definite quantitative correlation at the end of four minutes' incubation in dilutions greater than 1 : 40. Subsequent observations on serum confirmed this observation. Theoretically, therefore, if 1 cc. of a 1 : 50 dilution of plasma or serum inactivates or neutralizes 0.5 unit of thrombin in four minutes, undiluted plasma or serum should inactivate or neutralize fifty times as much, or 25 units. It was obvious from the amount of thrombin inactivated by serum and plasma in the previous studies that the antithrombic activity of diluted plasma is not a true index of the ability of undiluted plasma or serum to neutralize thrombin.

The following observations were made in an attempt to correlate the thrombin-neutralizing activity of diluted and undiluted serum and plasma (table 2). Such a correlation was advisable because of the technical difficulties in preparing purified thrombin in quantity. The antithrombic

activity of 10 specimens of diluted serum which had been incubated at 56 C. for ten minutes was determined, only those dilutions being used in which sufficient thrombin remained after four minutes of incubation at 28 C. to clot the added fibrinogen in thirty to thirty-five seconds. The calculated amounts of thrombin inactivated were between 15.5 and 27.0 units per cubic centimeter of serum. Various quantities of purified thrombin were then added to 1 cc. of each specimen of undiluted serum and the amounts determined that were neutralized in four minutes' incubation at 28 C. Regardless of the quantity of thrombin added, there was always a small amount remaining. Thus, the addition of small amounts (15 to 60 units) of thrombin did not give a true index of the antithrombic activity. Because of these findings, 100 to 140 units of thrombin was added to each cubic centimeter of the undiluted serum, and

TABLE 2.—*Variations in the Antithrombic Activity of Diluted and Undiluted Serum, with Calculation of the Average Correction Factor*

Source	Units of Thrombin Added per Cc.	Units of Thrombin Inactivated in 4 Minutes	Calculated Units of Thrombin Inactivated per Cc., Dilution Method	Correction Factor *
Normal human subject	100	86.0	16.5	5.21
Normal human subject	100	89.0	15.5	5.74
Normal human subject	100	88.0	16.5	5.33
Normal human subject	120	101.0	20.0	5.05
Rabbit	100	87.5	16.5	5.30
Rabbit	100	87.0	16.5	5.27
Rabbit	100	89.0	16.5	5.27
Rabbit	120	107.0	20.0	5.39
Rat (albino)	140	133.0	27.0	4.92
Rat (albino)	140	122.0	23.0	5.30

* The average correction factor was 5.28.

it was observed that from 86 to 133 units was inactivated in four minutes. Hence, to correlate the antithrombin of diluted and undiluted serum a correction factor of 5.28 was necessary, which was obtained by dividing the amount of thrombin neutralized by undiluted serum by the amount of thrombin calculated to be inactivated by the diluted serum.

METHOD FOR THE QUANTITATIVE DETERMINATION OF ANTITHROMBIN IN SERUM AND PLASMA

With these accumulated data from the studies on quantitative activity of antithrombin it has been possible to devise a test for the titration of antithrombin in serum and plasma.

Venous blood is obtained and either is allowed to clot or is mixed with one seventh of its volume of a 1.85 per cent solution of potassium oxalate. After centrifugation the hematocrit reading and the total volume of the oxalate blood are carefully recorded to permit correction for dilution by the oxalate. The plasma or serum is collected after centrifugation and incubated in a water bath for ten

minutes at 56 C. The precipitated fibrinogen of the plasma may then be removed by centrifugation. Serum does not require centrifugation. Dilutions of the serum or plasma of 1:40, 1:50 and 1:60 are made with physiologic solution of sodium chloride. To 15 drops of each dilution is added 1 drop of thrombin containing 1 unit. The mixture is then allowed to stand for four minutes before the addition of 5 drops of fibrinogen. The time of the appearance of the fibrin strands is then determined. The end point is quite distinct. The incubation temperature should be 28 to 30 C. For accurate readings only those dilutions in which the clotting time occurs between twenty-eight and thirty-five seconds are used.

On the basis of observed quantitative activity, 1 unit of antithrombin is defined as that amount which will inactivate 1 unit of thrombin in four minutes at 28 C. No variation in antithrombic unitage occurs between 25 and 37 C. if the thrombin is standardized at the same temperature utilized in the ultimate test. In calculating the number of units per cubic centimeter of plasma, correction is made for the oxalate dilution factor. No such correction is necessary for the serum. The denominator of the final dilution is then multiplied by the amount of thrombin

TABLE 3.—*Normal Levels of Antithrombin in the Blood of Human Subjects and Various Animals*

Source	Number of Subjects	Average Antithrombin, Units per Cc.	Range of Variation, Units per Cc.*
Normal human subject	34	90	74-115
Dog	15	87	74-105
Cat	5	96	79-117
Cow	11	93	82-119
Pig	4	103	101-109
Guinea pig	10	108	97-129
Rabbit	18	108	98-133
Rat (albino)	28	123	102-147

* Lowest and highest determinations.

inactivated and the result multiplied by the correction factor, 5.28. The answer obtained is in units of antithrombin per cubic centimeter of serum or plasma.

LEVELS OF ANTITHROMBIN IN BLOOD SERUM AND PLASMA

Blood was obtained from human subjects and various laboratory and domestic animals and the antithrombin of the serum or plasma determined by the method just described. In those cases in which the antithrombin was determined on both serum and plasma, the slight variations noted were within the range of experimental error.

The antithrombin level of the individual species was fairly constant. Human subjects have an average antithrombin level of 90 units per cubic centimeter. Dogs, cats and cattle have approximately the same antithrombin levels as human beings. The antithrombin level of guinea pigs, hogs and rabbits is slightly higher. The highest average unitage observed in the species studied was 123 units in the albino rat. It was interesting to note that calves consistently had slightly more antithrombin than cattle.

Daily observations were made on 1 normal human subject and a small series of dogs and rabbits. The antithrombin level of each subject was found to remain remarkably constant from day to day, variations of only ± 5 per cent having been recorded. All specimens of blood were obtained when the subject was in the fasting state, and no attempt was made to study the possible effect of various dietary measures.

The antithrombin and prothrombin concentrations were observed in a person with severe cirrhosis of the liver accompanied by spontaneous ecchymosis and bleeding gums. The plasma prothrombin was 25 per cent of normal, or 75 units per cubic centimeter, and the antithrombin was 95 units. In another patient with obstructive jaundice due to carcinoma of the head of the pancreas but with no clinical evidence of hemorrhage the prothrombin was 29 per cent of normal, or 87 units per cubic centimeter, and the antithrombin was 90 units per cubic centimeter. It is perhaps worthy of note that the hemorrhagic zone in hypoprothrombinemia occurs at the level at which the prothrombin unitage approximates or is lower than the antithrombin unitage.

COMMENT

There is little, if any, difference in the antithrombic activity of serum and plasma. This is surprising when one realizes that the antithrombin of rabbit serum has already inactivated from 270 to 300 units of thrombin. From these observations it seems justified to conclude that the normal antithrombin of blood is quantitatively altered little, if any, during the clotting process. Charles and Scott,[2] however, were able to obtain only a trace of heparin from serum, although they observed that plasma contained 185 units in crude form.

The exact chemical nature of antithrombin as it exists in normal serum and plasma is not known. Howell and Holt [1] have demonstrated both antithrombin and heparin in plasma. Landsberg [20] postulated that thrombin is probably adsorbed by serum proteins, just as rennin may be adsorbed by casein. Quick,[18] in a recent review of the subject, cited evidence for the identity of normal antithrombin of blood with serum albumin or with a closely related substance. The addition of heparin to albumin increases the ability of the latter to neutralize or inactivate thrombin. These observations have been confirmed by Ferguson.[5] Ziff and Chargaff [21] stated that the antithrombin is present in the more soluble portion of the albumin fraction. The ability of serum or plasma

20. Landsberg, M.: Studien zur Lehre von der blutgerinnung. Physikalisch-chemische Vorgänge in ihrer Bedeutung für die Thrombinwirkung, Biochem. Ztschr. **50**:245, 1913.

21. Ziff, M., and Chargaff, E.: The Mechanism of Action of Heparin, Proc. Soc. Exper. Biol. & Med. **43**:740-742, 1940.

to inactivate thrombin is destroyed by incubation for ten minutes at 66 to 67 C. The importance of the SO_3H group has been emphasized by Demole and Reinert.[22] Howell [23] reported that heparin is a complex compound containing uronic acids, calcium and sulfuric acids. Jorpes [24] has stated that chemically heparin is probably a polysulfuric ester of mucoitin. Chargaff, Bancroft and Stanley-Brown [25] have noted that all substances of high molecular weight active in the inhibition of blood clotting contain sulfur. The antithrombin of serum or plasma, as is probably true of antiprothrombin, requires the presence of two factors, an activator, such as heparin, and the precursor of antithrombin, designated by Howell as proantithrombin.

The effect of electrolytes on the coagulation of fibrinogen by thrombin has been reported by Glasko and Greenberg.[26] Anions inhibit coagulation by acting directly on thrombin, the antithrombic action being negligible for univalent ions and increasing strongly with the valency. The inhibiting action of polyvalent cations is due to an effect on the fibrinogen. Although potassium oxalate will inactivate thrombin, the mechanism invoked is entirely different from that affecting heparin. Howell and Holt [1] have shown that heparin activates the proantithrombin of plasma, resulting in a marked increase in antithrombin, but heparin has a weak antithrombic activity per se. That potassium oxalate does not activate proantithrombin has been demonstrated in these studies by the fact that in high dilutions there is no difference in the activity of antithrombin of oxalated plasma and that of plasma which has had the oxalate removed by dialysis. Although not reported in detail, experimental observations on the addition of heparin to plasma indicated a marked increase in antithrombin.

During the actual process of blood coagulation the prothrombin is converted to thrombin. Thrombin has two possible fates; either it may combine with the fibrinogen, which results in the production of fibrin, or it may be inactivated or neutralized by antithrombin, with the sub-

22. Demole, V., and Reinert, M.: Polyanetholsulfosaures Natrium, ein neues synthetisches Mittel zur Hemmung der Blutgerinnung, Arch. f. exper. Path. u. Pharmakol. **158**:211-218, 1930.

23. Howell, W. H.: The Purification of Heparin and Its Chemical and Physiological Reactions, Bull. Johns Hopkins Hosp. **42**:199-206, 1928.

24. Jorpes, E.: Heparin: Its Chemistry, Physiology and Application in Medicine, London, Oxford University Press, 1939.

25. Chargaff, E.; Bancroft, F. W., and Stanley-Brown, M.: Studies on the Chemistry of Blood Coagulation: II. On the Inhibition of Blood Clotting by Substances of High Molecular Weight, J. Biol. Chem. **115**:155-161, 1936.

26. Glasko, A. J., and Greenberg, D. M.: The Mechanism of the Inhibiting Effect of Electrolytes and Heparin on Blood Coagulation, Am. J. Physiol. **128**:399-407, 1940.

sequent formation of metathrombin. Mellanby [27] and Stromberg [28] have shown that when fibrinogen and thrombin are mixed together there is no decrease in the free thrombin until the very moment of coagulation, at which time a large portion suddenly disappears from the fluid. Gessard,[29] Foà [30] and Eagle [31] suggested that the thrombin is carried down with the clot, perhaps adsorbed onto its fibrils. Howell [32] has recovered thrombin from dried fibrin. Because of the lack of standardization of the various individual coagulation factors, no absolute quantitative studies were possible until the observations of Smith and his co-workers became available. The present observations have revealed that 1 mg. of fibrinogen causes the disappearance of 5.1 units of thrombin from solution. When fibrinogen is converted to fibrin in the presence of an excess of thrombin, the saturation point is rapidly reached in less than one minute and no further adsorption of thrombin occurs. Human plasma normally contains 2.2 to 4.5 mg. of fibrinogen per cubic centimeter. Thus, if plasma contains 300 units of potential thrombin in the form of its precursor prothrombin, only 11 to 22.5 units is adsorbed during the conversion of fibrinogen to fibrin, the remaining 277.5 to 289 units being neutralized or inactivated by the antithrombin.

With the data obtained in these observations, it has been possible to devise an adequate method for the quantitation of antithrombin. One cubic centimeter of human plasma or serum will inactivate or neutralize from 74 to 115 units of thrombin in four minutes by this method. Before the results of the present technic can be compared with those of procedures previously utilized an analysis of the methods is advisable. Such a comparison has only been possible since the purification and standardization of prothrombin and thrombin.[17] The methods previously used for the quantitative determinations of antithrombin have consisted of either adding less than 1 unit of thrombin to undiluted serum and observing the clotting time on the addition of fibrinogen after various periods of incubation [33] or adding a fraction of a unit or a few units to

27. Mellanby, J.: The Rate of Fibrin Ferment from Prothrombin by the Action of Thrombokinase and Calcium Chloride, J. Physiol. **51**:396-403, 1917.

28. Stromberg, H.: Methodisches über Blutgerinnung nebst Bemerkungen über das Wasen des Gerinnungsvorganges, Biochem. Ztschr. **37**:177-217, 1911.

29. Gessard, C.: Sur le fibrino-ferment, Compt. rend. Acad. d. sc. **150**:1617, 1910.

30. Foà, C.: Sulle leggi d'azione della trombina, Arch. di fisiol. **10**:479-500, 1912; abstracted, Zentralbl. f. Physiol. **17**:603, 1913.

31. Eagle, H.: Studies on Blood Coagulation: II. The Formation of Fibrin from Thrombin and Fibrinogen, J. Gen. Physiol. **18**:547-555, 1935.

32. Howell, W. H.: The Preparation and Properties of Thrombin, Together with Observations on Antithrombin and Prothrombin, Am. J. Physiol. **26**:453, 1910.

33. Howell.[10] Gasser.[11] Footnote 12.

plasma and observing the variation in the clotting time.[34] These methods were developed before the standardization technic of thrombin by Smith and his co-workers by which they defined 1 unit as that amount which will convert 1 cc. of fibrinogen to fibrin in fifteen seconds. In my observations it was noted that a fraction of a unit of thrombin may remain active for a considerable time when added to serum. Whether small quantities are protected in some manner so as to prevent their inactivation is as yet unexplained. It is evident that the addition of small quantities of thrombin to serum does not give a true index of the antithrombic activity, because when larger amounts are added 74 to 147 units may be neutralized in four minutes. The antithrombic activity of diluted serum or plasma is not a true index of the ability of undiluted serum or plasma to neutralize thrombin, a correction factor being necessary for interpretation. The observed antithrombin in these studies is more than a thousandfold greater than the amount previously described in serum or plasma by the majority of investigators.

In theory, deviations from the physiologic range of antithrombin should affect blood coagulation, thrombosis tending to occur when it is depleted, hemorrhages when it is increased. In fact, certain pathologic states have been observed in which an abnormal antithrombin level has been thought to be a significant contributing factor in the syndrome. The hemorrhagic state in hypoprothrombinemia usually occurs at those levels at which the prothrombin unitage is within the same range as that of the antithrombin unitage as measured by the present method. Several factors must be considered to explain the hemorrhagic diathesis in the hypoprothrombinemic person, namely, the quantity and convertibility of prothrombin[35] and the quantitative level of antithrombin. This observed phenomenon further explains the hemorrhagic diathesis which occurs as a result of hypoprothrombinemia due to various causes. As has been previously stated, the decreased plasma prothrombin per se does not explain the hemorrhagic tendency because 1 unit of thrombin may clot 1 cc. of fibrinogen in fifteen seconds, normal human plasma containing three hundred times this amount in the form of its precursor prothrombin. A decrease in antithrombin in postoperative thrombosis has been reported by Howell[10] and Bancroft, Kugelmass and Stanley-Brown.[36] It is well established that antithrombin can be increased in vivo by act administration of heparin and Witte's peptone. Anaphylactic

34. Quick.[13] Eagle, Johnston and Ravdin.[14]

35. (*a*) Warner, E. D.; Brinkhous, K. M., and Smith, H. P.: The Prothrombin Conversion Rate in Various Species, Proc. Soc. Exper. Biol. & Med. **40**:197-200, 1939. (*b*) Owen, C. A.; Hoffman, G. R.; Ziffrin, S. E., and Smith, H. P.: Blood Coagulation During Infancy, ibid. **41**:181-185, 1939.

36. Bancroft, F. W.; Kugelmass, I. N., and Stanley-Brown, M.: Evaluation of Blood Clotting Factors in Surgical Diseases, Ann. Surg. **90**:161-189, 1929.

shock results in either retarded or complete inhibition of blood clotting. Zunz and La Barre [37] explained this on the basis of an increase in antithrombin. Eagle, Johnston and Ravdin [14] have recently observed that anaphylactic shock in dogs and rabbits does not affect the prothrombin, fibrinogen, blood platelet or calcium level but does cause an extraordinary increase in antithrombin. This increase in antithrombin is due to an elevation in the heparin content of the blood. Jaques and Waters [38] have isolated crystalline heparin from the blood of dogs sensitized to horse serum and of dogs in anaphylactic shock produced during amytal anesthesia. Mills and Kitzmiller [12] have reported an increase in the antithrombin of the blood serum in patients with typhoid fever. Whether variations may be present in other pathologic states remains for future determination. Preliminary investigations seem to indicate that the antithrombin levels are not markedly altered in the majority of diseases.

SUMMARY

A quantitative test has been devised for the determination of the antithrombin of serum and plasma. One unit of antithrombin is defined as that amount which will inactivate 1 unit of thrombin in four minutes at 28 C.

Normal human plasma or serum contains an average of 90 units of antithrombin per cubic centimeter, which is as much as a thousand times the amount revealed by many of the previous methods.

There is little or no difference in the antithrombic activity of serum and that of plasma.

During the actual process of blood coagulation only a small portion of the thrombin is adsorbed onto fibrin, the remainder being inactivated or neutralized by the normal antithrombic activity of the serum.

The hemorrhagic tendency in hypoprothrombinemia is not, therefore, fully explained on the basis of decreased prothrombin and the variations in the conversion rate of prothrombin to thrombin. The hemorrhagic diathesis usually occurs when the prothrombin unitage approximates or is lower than the antithrombin unitage.

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THERAPEUTIC AND PROPHYLACTIC DETOXICATION OF SULFANILAMIDE, SULFAPYRIDINE AND SULFATHIAZOLE

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The process of aiding the body with sulfanilamide compounds in its fight against a specific bacterial invasion presents simultaneously to the body toxic chemicals which it must detoxify. The processes used by the body in detoxication are oxidation, reduction and conjugation. Frequently, oxidation and reduction precede conjugation. The compounds involved in conjugative detoxication are well known; no attempt will be made to review the extensive material as various excellent reviews on the subject are available.[1] There is a certain specificity exhibited in the processes of detoxication. The compounds involved are aminoacetic acid, choline, cystine, glucuronic acid, glutamine, sulfates, acetates and ornithine.

The chemotherapeutic efficacy of the sulfanilamide compounds—sulfanilamide, sulfapyridine (*2*-[paraaminobenzenesulfonamido]-pyridine), sulfathiazole (*2*-[paraaminobenzenesulfonamido]-thiazole, etc.—against infections caused by such organisms as streptococci, pneumococci and staphylococci is well established. These compounds are toxic per se, manifesting in human beings a variety of symptoms.[2]

From the Warner Institute for Therapeutic Research.

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A consideration of the literature on detoxication led us to attempt to decrease the toxicity of the sulfanilamide compounds without altering their chemotherapeutic efficacy. Generally, a reenforcement of the body's natural processes of detoxication was undertaken.

MATERIAL AND METHOD

Subjects.—A total of 7,000 white mice, 18 to 25 Gm. in weight, was used to study acute toxicities. Chemotherapeutic efficacy was tested on approximately 1,500 mice. Absorption speeds were determined in a series of 20 dogs. The degree of conjugation was determined in approximately 100 rats and 20 rabbits.

Acute Toxicities.—It was necessary to check the values given by various investigators for the oral lethal doses of the compounds used, sulfanilamide, sulfapyridine and sulfathiazole. It became evident from the outset that a multiplicity of factors affected the value, e. g., the suspending agent, the osmotic pressure of solutions in which the sulfanilamide compound was given, the diet of the mice before use, the hydrogen ion concentration of the aforementioned solutions, the weight of the mice and the usual biologic variation. Each of the controllable factors received appropriate attention in all experiments throughout the study.

All compounds used by the body in detoxication were regarded as potential detoxifying agents for sulfanilamide and its derivatives. Included among these compounds were cysteine, choline, aminoacetic acid, glutamine, sulfates, acetates and calcium glucuronate. A large number of other compounds of similar or unrelated structure were tried as potential detoxifying agents. All compounds tested are listed in table 1. Doses were usually administered at a level of 4 Gm. of sulfanilamide compound per kilogram of body weight. The detoxifying compound or mixture of compounds was fed at the same level, 4 Gm. per kilogram, except in specified instances. In the early experiments a 10 per cent solution of acacia was used as the suspending agent, but frequent unexplainable variation in control toxicity determinations led us to abandon acacia in favor of a 0.5 per cent solution of tragacanth. Volumes of material administered were held below 0.8 cc. It is possible to cause death in a series of animals by using 1.0 cc. doses of solutions which are osmotically active or which are not neutral in p_H. The sulfanilamide compound and the detoxifying chemical were administered in the same solution in the major portion of our experiments. A magnification of the detoxifying action is afforded if the detoxifying chemical is given several hours before the administration of the sulfanilamide compound. A long blunt syringe needle and a tuberculin syringe graduated in hundredths of a cubic centimeter were used throughout for oral administration. No group of animals less than 25 in number was considered, and no greater variation in weight than 4 to 6 Gm. in any set of 250 mice was permitted. The dietary regimen of the mice before use was controlled by maintaining them on a stock ration for several days and then placing them on an unpolished rice ration for three days before use. The latter diet is a maintenance ration, calorically adequate but inadequate nutritionally for other than maintenance. Diet before use is a most important factor. Diet variation can cause experimental results in control animals to vary by as much as 50 per cent. The solubilizing effect of possible variation in p_H was controlled by determinations of the absorption speeds of the compounds involved.

RESULTS

Sulfanilamide.—Table 1 presents a summary of the results obtained with various compounds and combinations of compounds in detoxifying sulfanilamide.

It is evident from these results that the toxicity of sulfanilamide can be significantly reduced by the simultaneous administration of even single doses of compounds used by the body in the general processes of detoxication.

TABLE 1.—*Protection Afforded by Various Physiologic Detoxifying Agents and Combinations of These Agents Against Acutely Toxic Doses of Sulfanilamide, Expressed in Terms of the Decrease in the Percentage Mortality Rate* *

Questionable Decrease or None	20% Decrease	40% Decrease
Glutamic acid	Sodium acetate	{Cysteine hydrochloride Glutamic acid
Nicotinic acid	Calcium gluconate	{Aminoacetic acid Ascorbic acid
Thiamine hydrochloride	Choline	{Calcium glucuronate Calcium glucuronate
Sodium sulfate		
Alanine	{Calcium glucuronate Ferrous chloride	
Serine		
Leucine		Calcium glucuronate
Isoleucine	Liquid yeast concentrate	
Valine	Methionine	{Cystine Aminoacetic acid
Mucic acid	Calcium aldobionate	{Calcium glucuronate Ascorbic acid
Levulose	Arabinic acid	
Aldohexoses	2-ketogluconic acid	Ascorbic acid
Dihydroxyacetone	Saccharolactone	
Lactic acid	Glutamine	
Pyruvic acid	Cysteine hydrochloride	
Saccharic acid	Aminoacetic acid	
Pectic acid	Isoascorbic acid	
Glyoxalic acid		
Sodium propionate		
Lecithin		
Cephalin		
Cholesterol		
Xanthine		
Adenine		
Guanine		
Acetaldehyde		
Ornithine		

* Compounds bracketed together were administered in combination.

In summary, the acute toxic effects of sulfanilamide in albino mice can be reduced by as much as 40 per cent by the simultaneous administration of single doses of compounds used by the body in detoxication. Calcium glucuronate was found to be the most efficacious single one of these compounds. A combination of aminoacetic acid, cystine, calcium glucuronate and ascorbic acid was observed to provide the most consistent and the greatest protection.

With this pronounced reduction in toxicity, the question immediately arises as to whether the chemotherapeutic efficacy has been reduced. To test this, the same solutions were used to protect white mice, 18 to 22 Gm. in weight, against a streptococcic infection. The therapeutic dose of sulfanilamide used was 2 Gm. per kilogram, given with and without the simultaneous administration of a detoxifying chemical or chemicals.

The detoxifying chemicals were also tested in the absence of the sulfanilamide for possible direct action. The products of bacterial metabolism are known to be extremely toxic; thus an obvious potentiality presented itself. The inoculum used was 0.1 cc. of an undiluted broth culture of Streptococcus haemolyticus. An attempt was made to hold this dose of the bacteria at such a level as to kill less than 100 per cent of the animals in the negative control series. The dose of sulfanilamide was then adjusted to afford a difference in death rate percentages of 20 to 40 or 50. The results of testing the chemotherapeutic efficacy of sulfanilamide in the presence of various detoxifying chemicals show conclusively that there is no decrease in the chemotherapeutic efficacy of sulfanilamide. On the contrary, there seems to be a slight but definite enhancement of the antistreptococcus activity. There was a difference in the percentage death rates of approximately 10 in the 3,000 mice used. Furthermore, an extension of the survival period is seen when the

TABLE 2.—*Absorption Speed of Sulfanilamide as Influenced by Detoxifying Chemicals* *

Hour	Dose: 1.0 Gm. of Sulfanilamide With 1.0 Gm. of Detoxifying Mixture	Dose: 1.0 Gm. of Sulfanilamide Without Any Detoxifying Mixture
1	3.79	3.96
2	6.74	5.46
3	10.03	7.65
4	8.13	6.86

* Values are expressed in milligrams of sulfanilamide per hundred cubic centimeters of blood and represent the average for 4 dogs.

detoxicant is given to mice with streptococcic infections. The detoxicant alone both extends the survival period of these mice and causes a slight decrease in the percentage of deaths due to the infections.

To check further the possibility that decreased absorption may have been a factor in the decreased toxicities observed, dogs were given doses of 1 Gm. of sulfanilamide, first without an added detoxifying agent, then with the detoxifying compound or mixture of compounds and finally again without the detoxifying chemical. The concentration of sulfanilamide in the blood was measured hourly by the method of Bratton and Marshall.[3] In each instance the three determinations were carried out on the same dog, in order to eliminate any factors varying from animal to animal. The results observed in the case of sulfanilamide are recorded in table 2.

Clearly, there is no decrease in the speed of absorption and the maximum concentration reached is not diminished when the detoxicant is given with the sulfanilamide. It is obvious, therefore, that the decreased

3. Bratton, A. C., and Marshall, E. K., Jr.: New Coupling Component for Sulfanilamide Determination, J. Biol. Chem. **128**:537, 1939.

toxicity of sulfanilamide given with one or more detoxifying chemicals cannot be traced to decreased absorption. The results would even indicate an increased absorption to the extent of approximately 10 per cent, which possibly may be a factor in the increased chemotherapeutic efficacy of sulfanilamide when administered with the detoxifying compounds.

In summary, we have demonstrated that the administration of sulfanilamide with a detoxifying chemical decreases the toxicity of sulfanilamide by as much as 40 per cent, increases its chemotherapeutic efficacy against Str. haemolyticus by 10 per cent and increases the speed of its absorption from the gastrointestinal tract by approximately 10 per cent.

Sulfapyridine.—Using 700 mice and the technic employed in the study of sulfanilamide, we found that the acute toxicity of sodium sulfapyridine was reduced by as much as 50 per cent by the simultaneous admission of detoxifying chemicals. Aminoacetic acid and ascorbic acid seemed to be the most effective single detoxifying chemicals. Again, to determine the effect on chemotherapeutic efficacy, we administered sodium sulfapyridine with and without a detoxifying mixture to animals with infections caused by pneumococci of type II. From the results of a study involving 750 mice, it can be concluded that there was a distinct shift in the percentage of deaths for each day. The presence of the detoxifying chemicals brought about a distinct prolongation of the survival period of the entire series. As in the case of sulfanilamide, the absorption speed of the sodium sulfapyridine with and without the detoxicant was measured in 6 dogs. The results showed that there is no decrease in the speed of absorption and the maximum concentration reached is not diminished when the detoxicant is given with the chemotherapeutic agent.

Sulfathiazole.—Five hundred mice were used in testing the activity of detoxifying chemicals in acute sodium sulfathiazole intoxication. Here, as in the case of sodium sulfapyridine, the number of deaths in the entire series was reduced by as much as 50 per cent. Cystine and aminoacetic acid seemed to be the two chemicals which individually exerted the greatest effect in combating sodium sulfathiazole intoxication. The chemotherapeutic efficacy against pneumococci of type II of sodium sulfathiazole given with and without the detoxifying chemicals was tested in a series of 750 mice. That the animals receiving detoxifying chemicals were protected was manifested in prolonged survival periods. The absorption speed was not altered by the concomitant administration of detoxifying chemicals.

COMMENT

The sulfanilamide compounds (sulfanilamide, sulfapyridine and sulfathiazole) must be detoxified by oxidation or reduction and subsequent conjugation. Acetylation of sulfanilamide has been demonstrated in the

human subject and in the rabbit by Marshall and associates,[4] and Harris and Klein [5] first demonstrated that it takes place in the liver. Acetylation both of sulfanilamide and of sulfapyridine was described for the cat by van Winkle and Cutting.[6] The liver was again designated as the site of detoxication. Conjugation seems to be the final and essential step in detoxication. Recoveries of between 50 and 95 per cent of ingested sulfanilamide have been reported,[7] and a portion of this amount is conjugated as the acetyl derivative. The question remains as to whether all ingested sulfanilamide can be accounted for by the excretion of free and acetylated forms or whether it is excreted partly in some other form, such as the glucuronide. This potentiality has been strengthened by the observation of Scudi and associates [8] that increased glucuronate excretion followed the administration of sulfapyridine, and more recently, Scudi [9] has demonstrated hydroxysulfapyridine in dog urine and has isolated a water-soluble glucuronate of hydroxysulfapyridine. James [10] raised the question of the exact manner in which sulfanilamide and acetylsulfanilamide exerted their toxic action. He suggested that while the acetyl derivative had a direct toxic action, sulfanilamide killed by a sudden withdrawal of acetate precursors in the body. The toxicity of sulfapyridine was also attributed to the withdrawal of acetate ions. He observed a significant decrease in the toxicity both of sulfanilamide and of sulfapyridine when sodium acetate was given at the same time. Our results with sulfanilamide, sulfapyridine and sulfathiazole confirm the observations of James, but the decrease in toxicity is less marked with sodium acetate than with certain other detoxifying chemicals. There is some evidence in the literature contraindicating the use of acetates with the sulfanilamide compounds. Lehr and co-workers [11] have demonstrated that

4. Marshall, E. K., Jr.; Cutting, W. C., and Emerson, K., Jr.: Acetylation of *p*-Aminobenzene Sulfonamide in Animal Organism, Science **85**:202, 1937.

5. Harris, J. S., and Klein, J. R.: Acetylation of Sulfanilamide by Liver Tissue in Vitro, Proc. Soc. Exper. Biol. & Med. **38**:78, 1938.

6. van Winkle, W., Jr., and Cutting, W. C.: Acetylation of Sulfanilamide and Sulfapyridine in the Cat, J. Pharmacol. & Exper. Therap. **69**:40, 1940.

7. Stewart, J. D.; Rourke, G. M., and Allen, J. S.: Excretion of Sulfanilamide, J. A. M. A. **110**:1885 (June 4) 1938. Scudi, J. V., and Ratish, H. D.: Urinary Excretion of Single Dose of Sulfanilamide, J. Lab. & Clin. Med. **23**: 615, 1938. Marshall, E. K., Jr.; Emerson, K., Jr., and Cutting, W. C.: Paraaminobenzene Sulfonamide: Absorption and Excretion; Method of Determination in Urine and Blood, J. A. M. A. **108**:953 (March 20) 1937.

8. Scudi, J. V.; Ratish, H. D., and Bullowa, J. G. M.: Increased Glucuronate Excretion Following Administration of Sulfapyridine, Science **89**:516, 1939.

9. Scudi, J. V.: On Urinary Excretion of Sulfapyridine, Science **91**:486, 1940.

10. James, G. V.: Effect of Administration of Acetate on Detoxication and Therapeutic Activity of Sulphanilamide, Biochem. J. **33**:1688, 1939; **34**:633, 1940.

11. Lehr, D.; Antopol, W., and Churg, J.: Massive "Acute" Precipitation of Free Sulfathiazole in the Urinary Tract, Science **92**:434, 1940.

acetylated sulfathiazole precipitated in massive amounts in the urinary tract, causing sudden death in mice. The bladders of these animals on occasion became completely filled with the white crystalline material. Increased acetylation might aggravate the tendency of sulfathiazole to precipitate in the bladder as the acetylated derivative. Furthermore, Marshall and associates [12] stated that acetylsulfanilamide is more toxic when administered parenterally than is sulfanilamide itself. We have attempted to decrease the amount of acetylation by forcing conjugation into other channels, such as the formation of glucuronate. The administration of a detoxifying mixture containing cystine, aminoacetic acid, calcium glucuronate and ascorbic acid has been demonstrated [13] to decrease the acetylation of sulfanilamide in the rat.

Klein and Harris [14] studied the acetylation of sulfanilamide in vitro. They showed that acetylation of this compound can occur in slices of liver and that the factor limiting the reaction in speed is the rate of acetate production by the tissue. Addition of acetate increases the amount of conjugation, as does the addition of substances giving rise to acetate in the tissue. Pyruvate, lactate and acetoacetate had a variable effect in increasing conjugation, but acetate was constant in its effect.

Southworth [15] was the first to observe the fall of the carbon dioxide–combining power of the blood of patients treated with sulfanilamide. Hartmann, Perley and Barnett [16] then suggested the use of sodium lactate and Lucas and Mitchell [17] suggested sodium bicarbonate to counteract this fall. Lactate and bicarbonate would both raise the carbon dioxide–combining power, but the lactate has the advantage of being an acetyl precursor. The use of acetate, according to the work of James,[10] during the administration of a sulfanilamide compound offers certain advantages in modifying its acute toxicity, and James's experiments show that it acts by preventing the metabolism of acetate precursors. Other substances, such as lactate and bicarbonate, have been suggested for counteracting a fall in carbon dioxide–combining power, but acetate has this effect and in addition provides acetyl radical directly. James [18] also

12. Marshall, E. K., Jr.; Cutting, W. C., and Emerson, K., Jr.: The Toxicity of Sulfanilamide, J. A. M. A. **110**:252 (Jan. 22) 1938.

13. Martin, G. J.; Rennebaum, E. H., and Thompson, M. R.: Inhibition of Conjugation of Sulfanilamide, J. Biol. Chem. **139**:871, 1941.

14. Klein, J. R., and Harris, J. S.: Acetylation of Sulfanilamide in Vitro, J. Biol. Chem. **124**:613, 1938.

15. Southworth, H.: Acidosis Associated with Administration of Paraaminobenzene Sulfonamide (Prontylin), Proc. Soc. Exper. Biol. & Med. **36**:58, 1937.

16. Hartmann, A. F.; Perley, A. M., and Barnett, H. L.: Study of Some of the Physiological Effects of Sulfanilamide: Changes in Acid Base Balance, J. Clin. Investigation **17**:465, 1938.

17. Lucas, C. C., and Mitchell, D. R.: Biochemical Study of Patients on Sulphanilamide Therapy, Canad. M. A. J. **40**:27, 1939.

18. James, G. V.: Isolation of Some Oxidation Products of Sulfanilamide from Urine, Biochem. J. **34**:640, 1940.

clearly demonstrated that oxidation is a primary detoxicating mechanism for the sulfanilamide compounds. Para-N-acetylhydroxylaminobenzenesulfonamide, parahydroxyaminobenzenesulfonic acid and paraaminophenol were isolated from the urine of patients treated with sulfanilamide. James stated that these compounds appear to be excreted in conjugation with sulfates and glucuronates. The paraaminophenol undergoes further changes and is excreted as a pigment.

It is desired to point out that the action of the detoxifying chemical or chemicals is not specific for the sulfanilamide compounds under consideration in this paper but rather is a manifestation of a reenforcement of the processes ordinarily used by the body in its defense against toxic chemicals, regardless of their nature. This type of therapeutic or prophylactic detoxication is equally effective against the products of the metabolism of the organisms which invade the body and the specific chemotherapeutic agents used in the treatment of diseases caused by these organisms. The problem of the detoxication of absorbed toxic chemicals is the same as the problem of the detoxication of toxic chemicals produced within the body by an invading parasite. The general process of detoxication follows three lines: oxidation, reduction and conjugation. Frequently, oxidation and reduction precede conjugation. The processes of oxidation and reduction of toxic chemicals are being subjected to intensive study, but it remains difficult to apply any practical means of influencing these processes. Vitamin therapy doubtless owes a great deal of its effectiveness to the reestablishment of an enzyme moiety and normal processes of oxidation and reduction which are concerned in detoxication. Thus, there is available at present but one process which can be used to facilitate detoxication, namely, the process of conjugation. In passive immunity the body's defense is reenforced by supplying additional preformed antibodies. In conjugative detoxication it is possible to supply the body with an increased amount of the raw materials used in the processes of detoxication.

SUMMARY

The acute toxicities of sulfanilamide, sulfathiazole and sulfapyridine were markedly reduced by the prophylactic and therapeutic use of physiologic detoxifying chemicals, such as cystine, aminoacetic acid, calcium glucuronate and ascorbic acid.

The chemotherapeutic efficacy of the sulfanilamide compounds when administered with detoxifying chemicals was maintained or enhanced.

The speed of absorption was increased when a sulfanilamide compound was administered with detoxifying chemicals.

Therapeutic and prophylactic detoxication offers tremendous potentialities for clinical application.

RENAL LESIONS FOLLOWING THE INTRAVENOUS INJECTION OF A HYPERTONIC SOLUTION OF SUCROSE

A CLINICAL AND EXPERIMENTAL STUDY

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Sucrose has been widely used for tissue dehydration in such conditions as cerebral edema associated with trauma,[1] chronic hypertension,[2] acute alcoholism,[3] status asthmaticus,[4] postoperative anemia,[5] edema of nephrosis and nutritional edema.[6]

The quantity and the concentration of sucrose given to patients have varied widely. Dyar and Matthew [7] gave 400 cc. of a 25 per cent solution intravenously. These investigators found no contraindication to the use

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1. Hahn, E. V.; Ramsey, F. B., and Kohlstaedt, K. G.: Clinical Experience in the Use of Sucrose Instead of Dextrose in the Osmotic Therapy of Increased Intracranial Pressure Occurring in Cases of Acute Brain Injury, J. A. M. A. **108**:773 (March 6) 1937. Jackson, H.; Dickerson, D., and Gunther, A.: The Reduction of Intracranial Pressure in Cerebral Injury by the Intravenous Use of Hypertonic Sucrose Solution, Ann. Surg. **106**:161, 1937. Gotten, N.: Head Trauma, J. A. M. A. **110**:1727 (May 21) 1938.

2. Murphy, F. D.; Hershberg, R. A., and Katz, A. M.: The Effect of Intravenous Injections of Sucrose Solution (50 Per Cent) on the Cerebrospinal Fluid Pressure, the Blood Pressure and Clinical Course in Cases of Chronic Hypertension, Am. J. M. Sc. **192**:510, 1936.

3. Hilton, J. P., and Alderson, D. M.: Sucrose Solution in the Treatment of Alcoholism and Confusional States, Rocky Mountain M. J. **35**:227, 1938.

4. Keency, E. L.: The Effectiveness of Intravenous Hypertonic Sucrose and Adrenalin in the Treatment of Status Asthmaticus, J. Allergy **9**:497, 1938.

5. Strahm, J. G., and Osgood, S. B.: Intravenous Sucrose as a Diuretic, Northwest Med. **35**:89, 1936. Helmholz, H. F.: Sucrose as a Diuretic, J. Lab. & Clin. Med. **25**:1180, 1940.

6. Lowenburg, H., and Nemser, S.: Intravenous Injection of Fifty Per Cent Solution of Sucrose in Edema, Arch. Pediat. **53**:762, 1936. Lowenburg, H., and Miller, A. B.: A Case of Nutritional Edema, ibid. **55**:297, 1938.

7. Dyar, E. W., and Matthew, W. B.: Use of Sucrose Preparatory to Surgical Treatment of Glaucoma, Arch. Ophth. **18**:57 (July) 1937.

of sucrose except perhaps the presence of markedly low renal function. Masserman [8] has given intravenously as much as 500 cc. of a 50 per cent solution of sucrose. He expressed the opinion that "comparatively large amounts of sucrose in the circulation are not toxic and cause no serious disturbances in the chemistry or cytology of the blood." Keith and Power [9] found "the total excretion of the various urinary constituents during a day in which sucrose is injected but slightly altered aside from changes that may be attributed to diuresis." Murphy and his associates [2] gave 300 to 500 cc. of a 50 per cent solution of sucrose to 21 patients with chronic hypertension. The patients improved, and no unfavorable results were noted from the treatment.

Cutler [10] apparently was the first to give any information as to the quantity of sucrose that may safely be administered to patients. He concluded from his histologic studies of human kidneys that sucrose may produce renal lesions when approximately 6 to 10 Gm. per kilogram of body weight is given in a week.

The renal lesions that may occur in man after the injection of sucrose have been described by Helmholz,[11] Cutler,[10] Anderson and Bethea [12] and Anderson.[13] The pathologic process has been nicely described and well illustrated by the two last-named authors. The epithelial cells in the convoluted portion of the renal tubules become markedly swollen, and the cytoplasm is foamy. This change has been observed both as a focal and as a diffuse lesion.

In reviewing the clinical cases reported by Anderson and Bethea [12] and Anderson [13] we have been impressed with the fact that other investigators have given more sucrose to their patients without the development of any renal lesions. Anderson [13] discussed the probability that "pre-existing renal lesions may enhance the effects of sucrose on

8. Masserman, J. H.: Effects of the Intravenous Administration of Hypertonic Solutions of Sucrose, Bull. Johns Hopkins Hosp. **57**:12, 1935.

9. Keith, N. M., and Power, M. H.: The Urinary Excretion of Sucrose and Its Distribution in the Blood After Intravenous Injection into Normal Men, Am. J. Physiol. **120**:203, 1937.

10. Cutler, H. H.: Effect of Sucrose on the Kidney, Proc. Staff Meet., Mayo Clin. **14**:318, 1939.

11. Helmholz, H. F.: Renal Changes in the Rabbit Resulting from Intravenous Injection of Hypertonic Solution of Sucrose, J. Pediat. **3**:144, 1933.

12. Anderson, W. A. D., and Bethea, W. R., Jr.: Renal Lesions Following Administration of Hypertonic Solutions of Sucrose, J. A. M. A. **114**:1983 (May 18) 1940.

13. Anderson, W. A. D.: Sucrose Nephrosis and Other Types of Renal Tubular Injuries, South. M. J. **34**:257, 1941.

the tubules so that changes are produced from relatively small amounts." He asserted:

> Sucrose nephrosis was found to be severe in kidneys involved by marked vascular disease. It may well be that interference with renal circulation by vascular change, or a similar effect produced by thickening of glomerular basement membrane, will markedly enhance the damaging effect of sucrose. Particularly if there was a reduction in filtration rate, it would operate to prolong the exposure of the epithelial cells to the sucrose in the filtrate.

In considering Anderson's explanation of the effect of previous renal disease on the development of tubular lesions following the injection of sucrose, it must be remembered that Murphy, Hershberg and Katz [2] gave 300 to 500 cc. of a 50 per cent solution of sucrose to 21 patients with chronic hypertension. This treatment relieved such symptoms as headache, vomiting, vertigo, twitching and dizziness. No unfavorable results were observed in any of the 21 patients.

Lindberg and associates [14] gave 200 cc. of a 50 per cent solution of sucrose to 15 persons without any renal damage.

> In no case was there evidence of marked impairment of renal function. An occasional specimen of urine after the injection showed a 1 plus reaction for albumin or hyaline casts. The urea clearance remained essentially stationary, and in some cases it was increased by 3 to 5 per cent . . . Five patients with known renal damage were similarly studied. In these, too, the urine and urea clearance showed no significant changes as a result of the injection of 200 cc. of sucrose intravenously.

The following factors have impressed us in reviewing the autopsy reports on a group of patients who were given sucrose: (1) the quantity of sucrose administered; (2) the state of dehydration at the time the sucrose was injected, and (3) the fluid intake and output during the period in which sucrose was given. The following case reports will illustrate these factors.[15]

REPORT OF SIXTEEN CASES

Case 1.—A white man aged 64 became comatose two days before admission. He lived four hours after hospitalization and only three hours after the intravenous injection of 200 cc. of a 25 per cent solution of sucrose.

Generalized arteriosclerosis, with an occlusion in the left coronary artery and a recent myocardial infarct, was evident at autopsy. Scars were also present

14. Lindberg, H. A.; Wald, M. H., and Barker, M. H.: Renal Changes Following Administration of Hypertonic Solutions, Arch. Int. Med. **63**:907 (May) 1939.

15. Cases 1, 2, 3, 4, 6, 7 and 10 have been included in previous papers by Anderson and Bethea [12] and Anderson.[13] Dr. W. D. Forbus, professor of pathology, Duke University, has permitted us to include cases 13 to 16. Dr. A. J. Gill, of Duke University, summarized the histories of these cases.

in the myocardium. The cavities of the heart were dilated, and the viscera were congested. Bronchopneumonia was present. The renal arteries were sclerotic. The epithelial cells in the convoluted portion of the tubules were swollen, and their cytoplasm was foamy.

The three hour interval between the injection of sucrose and death was the shortest period which has elapsed between injection of sucrose and postmortem study of the kidneys in this series. It illustrates the rapidity with which the renal lesion may develop. The histologic changes in this case were similar to those observed in cases in which death occurred several days after the injection of sucrose. The fluid intake no doubt was small before hospitalization, since the patient was unconscious. The amount of fluid lost through the skin should have been large, since he died in June. This case illustrates the role that dehydration may play in the subsequent development of tubular changes following the injection of sucrose.

There was no evidence from the histologic studies that a pathologic condition existed in the kidneys before the patient received the sucrose.

Case 2.—A Negro aged 67 gave a history of a severe headache of three months' duration. He became drowsy, and the muscles of the upper extremities began to twitch. He became unconscious three hours before admission and remained comatose until death. He died thirty-six hours after hospitalization.

The temperature on admission was 104 F., the pulse rate 130 per minute, the respiratory rate 32 to 40 per minute and the blood pressure 140 systolic and 85 diastolic. The spinal fluid pressure equaled 218 mm. of water. The specific gravity of the urine was 1.023, and the albumin present gave a 2 plus reaction. Occasional white blood cells and casts were found in each high power field. The blood contained 30 mg. of nonprotein nitrogen per hundred cubic centimeters. The numbers of white cells and red cells and the quantity of hemoglobin in the blood were normal.

During the thirty-six hours of hospitalization the patient was given a total of 1,000 cc. of a 25 per cent solution of sucrose intravenously and 14 cc. of a 50 per cent solution of magnesium sulfate intramuscularly.

Generalized arteriosclerosis was observed at autopsy. Small hemorrhages were present in the endocardium. The prostate gland was hypertrophied. Bronchopneumonia was present. The cortex of the kidneys was slightly scarred. Apparently every epithelial cell in the convoluted portion of the renal tubules was markedly swollen, and the cytoplasm was foamy. An albuminous precipitate was present in Bowman's space in some of the glomeruli and in the lumen of some of the tubules.

This patient apparently had some renal insufficiency before receiving the sucrose. There are not enough data to determine the state of hydration.

Case 3.—A white man aged 74 gave a typical history and showed the classic signs of myocardial failure on admission. A systolic murmur could be heard over the entire precordium. The temperature was 96.4 F., the respiratory rate 30 per minute, the pulse rate 88 per minute and the blood pressure, 130 systolic and 70 diastolic. The patient lived only a day and a half in the hospital. On admission the blood contained 30 mg. of nonprotein nitrogen per hundred cubic centimeters. Eleven and a half hours before death he was given 200 cc. of a 25 per cent solution of sucrose intravenously.

Healed rheumatic endocarditis of the aortic and the mitral valve, with stenosis, was evident at autopsy. The heart was hypertrophied, and the cavities

were dilated. Chronic passive congestion and generalized arteriosclerosis were present. Precipitated protein was present in Bowman's space in some of the glomeruli. Apparently, every epithelial cell in the convoluted portion of the renal tubules was swollen, and the cytoplasm was foamy.

The presence of a normal amount of nonprotein nitrogen in the blood on admission would suggest a relatively normally functioning kidney. Protein in Bowman's spaces, observed histologically, may have resulted from the chronic passive congestion. This case illustrates the diffuse tubular injury that may follow the intravenous injection of sucrose. It is the only case in this series in which the patient had severe edema at the time the sucrose was given.

CASE 4.—A white woman aged 69 became unconscious at 7:30 a. m., while eating breakfast, and thereafter was unable to speak. She vomited dark, blood-like material on several occasions. Thirty minutes after this accident the patient was admitted to the hospital. She was unconscious but apparently not completely paralyzed. The temperature was 97 F., the pulse rate 96 per minute, the respiratory rate 36 per minute and the blood pressure 145 systolic and 68 diastolic. The spinal fluid was bloody. She was given 2 cc. of a 50 per cent solution of magnesium sulfate intramuscularly and 100 cc. of a 25 per cent solution of sucrose intravenously. Death occurred fourteen hours after the cerebral accident and twelve hours after the intravenous injection of sucrose.

A ruptured aneurysm at the bifurcation of the right internal carotid artery was evident at autopsy. Blood had dissected into the ventricles of the brain. The cavities of the heart were dilated, and the viscera showed the changes associated with venous congestion. The epithelial cells in the convoluted portion of the renal tubules were swollen and granular. The cytoplasm of these cells was not as "foamy" as has been observed in other patients given sucrose. There were a few scars in the cortex and focal collections of lymphocytes in the interstitial tissues.

The quantity of sucrose given to this patient was only half that given to others in whom tubular lesions occurred. Nothing in the kidneys indicated previous renal disease. The tubular changes which may have resulted from the administration of sucrose were indefinite. This case is important only because of the small quantity of sucrose which was administered and the absence of definite tubular lesions attributable to the sucrose.

CASE 5.—A white man aged 68 fell unconscious approximately three hours before admission to the hospital. After a few minutes consciousness returned; however, within thirty minutes he again lapsed into coma. He was paralyzed on the right side of the body. Coma persisted, and death occurred forty-eight hours after admission to the hospital.

The patient was given 84 ounces (2,484 cc.) of milk and fruit juices through a Levin tube during hospitalization. Twelve hours before death he was given 100 cc. of a 25 per cent solution of sucrose intravenously.

A generalized arteriosclerotic process, with a cerebral hemorrhage in the area of the right internal capsule, was apparent at autopsy. The heart was hypertrophied, and the cavities were dilated. The viscera were congested. Arteriosclerosis was present both in the large and in the small arteries of the kidneys. The arterioles also were thickened. Scars and collections of lymphocytes were present in the interstitial tissue of the renal cortex. The epithelial cells lining the convoluted portion of the tubules were swollen, and the cytoplasm was finely

granular. The changes resembled those observed in cloudy swelling more than they did the tubular lesions that occur after the injection of sucrose.

The tubular lesions in this case may have been the result of the administration of sucrose; however, at this time there is nothing by which we can differentiate them from cloudy swelling. There are insufficient data to determine the degree of renal function and the state of hydration at the time the sucrose was given.

CASE 6.—A Negro aged 33 gave a history of severe, generalized headache and dizziness of three months' duration, spots before the eyes and progressive failure of vision. Massive hemorrhages, papilledema and exudates were present in the retina.

The blood pressure was 220 systolic and 163 diastolic, the temperature 99 F., the pulse rate 100 per minute and the respiratory rate 32 per minute. The urine had a specific gravity of 1.016 and gave a 2 plus reaction for albumin. Microscopic examination of an uncentrifuged specimen showed 5 to 8 white cells, an occasional red cell and 1 granular cell per high power field. The spinal fluid pressure equaled 300 mm. of water. There was moderate anemia. The hemoglobin content was 11.5 Gm. per hundred cubic centimeters of blood. On the third day in the hospital the patient became nauseated and vomited; he appeared lethargic and progressively became comatose. He died eight hours after the last injection of sucrose. The following data appear to be important:

Date	Course of Illness	Magnesium Sulfate, Orally, Oz.	29% Solution of Sucrose, Intravenously, Cc.	5% Solution of Dextrose, Intravenously, Cc.	Nonprotein Nitrogen in Blood, Mg. per 100 Cc.	Output of Urine, Cc.
5/2/39	Admission	...	...	.....	...	.....
5/3/39	.....	1.5	...	.....	75	1,600
5/4/39	.....	...	...	2,000	85	950
5/5/39	.....	1.5	750	.....	...	3,800
5/6/39	.....	...	...	.....	...	600
5/7/39	.....	1.5	...	500	...	1,300
5/8/39	Death	...	500	1,500	130	700

Severe, malignant arteriosclerosis was evident at autopsy. The heart was hypertrophied, and the cavities were dilated. Chronic passive congestion of the viscera and bronchopneumonia were present. The epithelial cells of many of the renal tubules were swollen, and the cytoplasm was foamy. The cells lining other convoluted tubules were small and stained deeply with hematoxylin. These did not show the swelling that may accompany the injection of sucrose.

The clinical and pathologic findings indicated severe nephritis, with renal insufficiency. This was present when the patient was admitted. The quantity of sucrose and magnesium sulfate given to this man apparently was sufficient to produce some dehydration. The most interesting pathologic process observed in this case resulted from the distribution of the renal lesions. The low cuboidal cells lining the convoluted portion of some renal tubules were not affected, while corresponding cells in other tubules were swollen and the cytoplasm was foamy.

CASE 7.—A Negress aged 60 complained of a sharp pain in the right temporal region on the day preceding admission to the hospital. At 2 a. m. on the day of admission she was found unconscious in bed. She was admitted to the hospital sixteen hours afterward. Coma was present and persisted until death, which

occurred twelve hours later. All the muscles were spastic. The reflexes were hyperactive in the upper extremities. The temperature was 101 F., the pulse rate 120 per minute, the respiratory rate 38 per minute and the blood pressure 200 systolic and 120 diastolic. The urine gave a 4 plus reaction for albumin. On admission the patient was sweating profusely. Her husband stated that she also sweated much during the night.

The patient was given 4 cc. of a 50 per cent solution of magnesium sulfate intramuscularly and 250 cc. of a 25 per cent solution of sucrose intravenously immediately after admission to the hospital. She died eleven hours after the injection of the sucrose.

Necropsy showed generalized arteriosclerosis and a cerebral hemorrhage into the right internal capsule. The blood had dissected into the ventricles. The heart was hypertrophied. There was an extensive arteriolosclerotic process in the kidneys. Some of the glomeruli were fibrosed. Areas of fibrosis and foci of lymphocytes were present in the cortex. In some focal areas the convoluted portion of the tubules were swollen and the cytoplasm was foamy. In other areas the epithelial cells were small and pyknotic.

There was both clinical and pathologic evidence of renal insufficiency. It was present preceding the injection of the sucrose. The absence of tubular lesions in those portions of the convoluted tubules in which the cells appeared to be of the regenerative type was interesting in view of the fact that a similar process was observed in cases 6 and 8. This patient, no doubt, was dehydrated on admission, since she had been unconscious for about sixteen hours. There was also a history of profuse sweating.

CASE 8.—A Negro aged 65 was paralyzed on the left side of the body on admission. No history was obtained. The spinal fluid was xanthochromic. The temperature was 99 F., the pulse rate 66 per minute, the respiratory rate 18 per minute and the blood pressure 134 systolic and 100 diastolic. The Kahn reaction of the blood was negative. A specimen of urine obtained by catheterization had a specific gravity of 1.024 and showed neither albumin nor sugar. The blood contained 30 mg. of nonprotein nitrogen per hundred cubic centimeters. There was severe anemia.

On the seventh day before his death the patient was given 100 cc. of a 50 per cent solution of sucrose intravenously and on the following day an additional 50 cc. Clinical signs of bronchopneumonia developed on the third day before death. The urinary output for six of the seven days preceding death was as follows: seventh day, 550 cc.; sixth day, 300 cc.; fifth day, 850 cc.; fourth day, 550 cc.; third day, 440 cc., and second day, 1,300 cc.

Generalized arteriosclerosis was evident at autopsy. A hemorrhage in the area of the internal capsule on the right side had ruptured into the lateral ventricle. Bronchopneumonia was present. Some of the glomeruli were fibrosed. The walls of a few of the arterioles were thickened. Hyaline casts were present in the lumens of some of the tubules. The epithelial cells in the convoluted tubules in focal areas of the kidneys were swollen, and the cytoplasm was foamy.

There was little if any evidence from either the clinical or the autopsy observations to indicate that renal disease existed at the time the patient was admitted to the hospital. The urinary output was low. This apparently was the result of a low intake of fluid. The tubular lesions were characteristic of those that may follow the injection of sucrose. The focal distribution of these lesions was interesting, since they occur often uniformly throughout the kidneys.

CASE 9.—On admission to the hospital a Negro aged 65 gave a history of severe headaches, occasional attacks of dizziness and pain in the back of three weeks' duration. The muscles of the upper extremities and the shoulders contracted involuntarily. The blood pressure was 270 systolic and 140 diastolic. A specimen of urine obtained by catheterization had a specific gravity of 1.012 and gave a 3 plus reaction for albumin. Microscopic examination of an uncentrifuged specimen showed 3 to 5 white cells per high power field. The white cell count was 21,100 and the red cell count 3,890,000 per cubic millimeter. There was 9.5 Gm. of hemoglobin per hundred cubic centimeters of blood. The Kahn reaction was negative.

The patient became stuporous and then comatose. He died six days after admission.

The following pertinent data were recorded day by day:

Date	Course of Illness	50% Solution of Magnesium Sulfate, Intramuscularly, Cc.	25% Solution of Sucrose, Intravenously, Cc.	Solution of Dextrose			Nonprotein Nitrogen in Blood, Mg. per 100 Cc.	Output of Urine, Cc.
				Amount, Cc.	Concentration	Route *		
4/22/39	Admission	16	200	2,000	5%	I.V. S.C.	166	2,800
4/23/39	.....	16	500	4,000	5%	I.V. S.C.	...	3,400
4/24/39	.....	8	950	4,000	5%	I.V. S.C.	...	5,150
4/25/39	.....	12	750	3,000	5% 10%	S.C. I.V.	...	2,600
4/26/39	.....	8	400	3,000	5% 10%	S.C. I.V.	107	2,600
4/27/39	.....	4	600	2,000	5% 10%	I.V. S.C.	...	400
4/28/39	Death	8	225	2,000	5% 10%	I.V. S.C.	...	...

* I.V. indicates intravenous; S.C., subcutaneous.

Autopsy revealed hypertrophy of the prostate gland, acute urethritis, acute cystitis and acute and chronic pyelonephritis. Pulmonary emboli and bronchopneumonia were present in both lungs. Arteriosclerotic nephritis was present, with red blood cells and albumin in the lumens of the renal tubules. Essentially every epithelial cell in the convoluted portion of the tubules was swollen, and the cytoplasm was foamy.

There was definite renal insufficiency at the time the patient was admitted to the hospital. The daily intake and output of fluid apparently were sufficient. The quantity of sucrose (3,625 cc.) that the patient received during a period of seven days apparently was too much regardless either of the fluid balance or of the state of renal function.

CASE 10.—A Negress aged 15 was approximately nine months pregnant. There was some edema of the feet and ankles. She gave a history of headaches for four or five months. A week before admission she had "blind staggers." Her family stated that the patient had had at least twenty-five convulsions on the day of admission. She was comatose, and edema of the lungs was present at the time of hospitalization.

The urine had a specific gravity of 1.009, gave a 3 plus reaction for albumin and a positive reaction for acetone and contained white and red blood cells. On

two occasions the blood level of nonprotein nitrogen was 26 and 30 mg. per hundred cubic centimeters. The white cell count was 21,000; the red cell count was 3,200,000, and the hemoglobin content was 9.4 Gm. per hundred cubic centimeters of blood.

A stillborn baby was delivered a few hours after admission. The following day the patient's condition was critical. The renal output was small. There were some edema of the extremities at this time and extensive edema of the face. During the patient's stay in the hospital the blood pressure varied markedly from time to time from a low level of 90 systolic and 30 diastolic to a high level of 180 systolic and 110 diastolic. Fever was constantly present, and the temperature varied from 101 to 108 F. Clinical signs of bronchopneumonia developed on the day preceding death.

The fluid intake and output and the quantity of sucrose and magnesium sulfate that this patient was given during hospitalization are tabulated as follows:

Duration of Medication	Fluid Intake, Cc.	Fluid Output, Cc.	Sucrose, 25 per Cent Solution, Cc.	Other Dehydrating Medication
1 day	850	450	50	20 cc. 20 per cent magnesium sulfate, I.V.
2 days	300	250	1,500	6 oz. saturated solution of magnesium sulfate, stomach tube
3 days	2,000	300	600	
4 days	3,000	300	.....	
5 days	3,240	250	.....	20 cc. 50 per cent magnesium sulfate, I.M.
6 days	1,650	250	.....	
7 days	3,050	700	300	
8 days	3,000	420	.....	
9 days	2,000	300	.....	
10 days	1,550	.....	.....	
Total	20,640	3,220+	2,450	

The anatomic diagnosis was as follows: eclampsia and spontaneous delivery; puerperal infection and extensive necrosis of the cervix and the uterine mucosa (a smear of material from the uterus showed Bacillus welchii, Bacillus coli, Staphylococcus aureus and a nonhemolytic streptococcus); fresh thrombi in the blood vessels and the wall of the uterus and in the broad ligament; embolic abscesses in the lungs; acute fibrinous pleurisy in the left lung; focal areas of necrosis in the mucosa of the colon; petechiae in the mucosa of the duodenum; an acute ulcer in the duodenum (traumatic?); hydropic degeneration of the epithelial cells of the kidneys (due to sucrose); decubitus ulcers over the skin of the body; surgical incisions over blood vessels in several areas of the body, and septicemia (causative organisms, Staph. aureus and a nonhemolytic streptococcus).

Essentially every epithelial cell in the convoluted portion of the tubules was swollen, and the cytoplasm was foamy. The lumens of the tubules were occluded. A small amount of albumin was present in some of Bowman's spaces. Hyaline droplets were present in the cytoplasm of some of the tubular epithelial cells. There were no significant changes in the glomeruli.

It is difficult to determine the role that each of the several disease processes may have played in this patient. It may be said, however, that the function of

the kidneys was abnormal on admission, as shown by the specific gravity of the urine and the excessive excretion of albumin. The effect of the bacterial infection would certainly help to lower the renal reserve. It is of interest to observe the small fluid output and the large intake beginning on the third day. Swelling of the epithelial cells, as a result of the injection of the sucrose on the first and the second day of hospitalization, may have been sufficient to reduce the renal output. Edema became more extensive during hospitalization.

CASE 11.—A Negress aged 8 gave a history of headache, stiffness of the neck and paralysis of the right side of the body of one week's duration and paralysis of the left side of two days' duration. She had been unable to swallow any food during the past four days and was unable to talk at the time of admission. The spinal fluid showed a trace of sugar and contained 660 mg. of chlorides per hundred cubic centimeters. There were 250 white cells per cubic centimeter of spinal fluid. These were primarily lymphocytes. Cultures of the spinal fluid were sterile. The white cell count was 6,500; the red cell count was 3,050,000, and the hemoglobin content was 10 Gm. per hundred cubic centimeters. The urine was normal. The patient died three days after admission to the hospital. The diagnosis was tuberculous meningitis.

This patient was given sucrose intravenously, and a series of determinations of the nonprotein nitrogen were made. The following data show the amount of fluid the patient received during the period of observation and the nonprotein nitrogen content of the blood at frequent intervals:

Date	Hour	Course of Illness	Administration of Fluids			Nonprotein Nitrogen in Blood, Mg. per 100 Cc.
			Fluid	Amount	Method	
9/15/40	.....	.....	Milk, fruit juice 5% solution of dextrose	900 cc. 2,000 cc.	Levin tube	..
9/16/40	.....	.....	5% solution of dextrose Milk, fruit juice	1,000 cc. 640 cc.	.....	..
	2:15 p.m.	.....	25% solution of sucrose	100 cc.	Intravenous injection	27
	4:30 p.m.	.....	Orange juice	240 cc.	.....	..
	4:45 p.m.	.....	5% solution of dextrose	1,000 cc.	Intravenous injection	..
	8:00 p.m.	.....	Water	6 oz.	Levin tube	31
9/17/40	7:30 a.m.	.....	5% solution of dextrose	2,000 cc.	Intravenous injection	24
			Fruit juice, milk Water	220 cc. 5 oz.	Levin tube Levin tube	
9/18/40	7:30 a.m.	.....	Milk, fruit juice	720 cc.	Levin tube	..
	5:25 p.m.	Death				

The amount of sucrose given to this child was only half the quantity that produced renal lesions in some of the adults included in this series. There was no evidence of renal injury either before or after the injection of sucrose. The fluid intake apparently was adequate.

CASE 12.—A Negress aged 27 gave a history of headache of nine days' duration. The spinal fluid pressure was increased. There were 600 white cells, with 92 per cent lymphocytes, per cubic centimeter of spinal fluid. The fluid contained 30 mg. of sugar, 680 mg. of chlorides and 82 mg. of total protein per hundred cubic centimeters. The white cell count was 19,000 and the red cell count only 3,220,000. The urine was normal. A diagnosis of tuberculous meningitis was made. The

patient was given sucrose for its possible effect on the retention of nonprotein nitrogen in the blood. The following data show the quantity of fluids given:

Date	Hour	Course of Illness	Administration of Fluids			Nonprotein Nitrogen in Blood, Mg. per 100 Cc.
			Fluid	Amount, Cc.	Method	
9/14/40	.....	.....	10% solution of dextrose	1,000	.....	..
9/15/40	1:25 p.m.	.....	25% solution of sucrose	100	Intravenous injection	..
	1:35 p.m.	.....	10% solution of dextrose	1,000	Intravenous injection	..
9/16/40	9:50 a.m.	.....	10% solution of glucose	1,000	Intravenous injection	..
	2:30 p.m.	.....	25% solution of sucrose	100	Intravenous injection	23
	8:00 p.m.	.....	.....	.....	.....	33
9/17/40	8:00 a.m.	.....	10% solution of dextrose	1,000	Intravenous injection	33
9/18/40	8:00 a.m.	.....	.....	.....	.....	24
9/19/40	.....	.....	Liquids	.....	Oral administration	..
9/20/40	.....	.....	Liquids	.....	Oral administration	..
9/21/40	.....	.....	.....	.....	.....	25
	8:00 a.m.	.....	.....	.....	.....	28
9/27/40	.....	Death				

The quantity of fluid that this patient received during medication with sucrose was apparently adequate. There was no evidence of renal insufficiency either before or after the injection of sucrose. Each injection of sucrose given to this patient was only half that given to other adults in this series in whom tubular lesions developed.

Case 13.—A white man aged 38 gave a history of a severe headache of two weeks' duration. The patient was semicomatose, mentally confused and extremely dehydrated. He was given 200 cc. of a 50 per cent solution of sucrose and within twenty minutes was able to answer questions intelligently.

There were signs of increased intracranial pressure. A diagnosis of either a brain tumor or a brain abscess was made. On the day following admission he was given 50 cc. of a 50 per cent solution of sucrose at 3:30 p. m. and a second injection of 200 cc. at 10 p. m. A cerebral abscess caused by Pneumococcus type III was drained on the third day of his stay in the hospital. On this same day he was given 200 cc. of a 50 per cent solution of sucrose, and an additional 100 cc. of sucrose solution was administered on the fourth day of hospitalization.

On the third and the fourth day of hospitalization he was also given 200 cc. of a 50 per cent solution of dextrose and 900 cc. of a 10 per cent solution of dextrose. Antipneumococcus serum, sulfamethylthiazole (2-[paraaminobenzenesulfonamido]-4-methylthiazole) and sodium sulfapyridine (the sodium salt of 2-[paraaminobenzenesulfonamido]-pyridine) were given after the evacuation of the abscess. Fluids were not forced because of the patient's condition. The fluid intake apparently reached its low point, 1,204 cc., on the fourth day of hospitalization. The patient died on the fifth day of hospitalization.

Essentially every epithelial cell lining the convoluted portion of the renal tubules was swollen. The cytoplasm was foamy. Some of these cells showed a greater

swelling than did others in corresponding portions of the tubules. There were a few scars in the kidney.

The patient was dehydrated at the time he was given the first injection of sucrose. A hypertonic solution of sucrose was administered on each day of hospitalization. Death occurred on the fifth day. The renal lesions were similar to those observed in rabbits given sucrose during a period of restricted fluid intake.

CASE 14.—A white woman aged 48 gave a history and presented the physical signs of an acoustic nerve tumor. At operation a large tumor was found and was resected piecemeal. The postoperative course was good until the third day. At this time the pulse and respirations became rapid and irregular. It was considered likely that some edema had developed in the region of the medulla and cerebellum. An intravenous injection of 200 cc. of a 50 per cent solution of sucrose was given at this time. The patient died eighteen hours later.

The patient was given several blood transfusions and an average daily injection of 1,500 cc. of saline solution.

Apparently every epithelial cell in the convoluted portion of the renal tubules was swollen. The cytoplasm was granular. Frequently these granules in the cytoplasm were widely separated by clear spaces. The lumens of the tubules were decreased in size, and some precipitated albumin was present in the tubules and in Bowman's spaces.

The renal lesions may be confused in this case with those of advanced cloudy swelling; however, they are consistent with the changes that occur in the rabbit after the injection of sucrose. Lesions such as these apparently represent a "physiologic-pathologic" process in a cell that may readily revert to normal function.

CASE 15.—A Negro aged 25 gave a history of headaches and convulsive seizures during the past five years. A diagnosis of an intracranial neoplasm was made, and at operation a large parasagittal meningioma was removed.

The patient was given several transfusions immediately after the operation. Frequent injections of solutions of dextrose in concentrations of 5 and 10 per cent were given intravenously. The fluid intake appears to have been adequate, especially during the later days of life.

Thirteen days after operation the patient was given 100 cc. of a 50 per cent solution of sucrose intravenously. On the twenty-third day after operation he was given 200 cc. of a 50 per cent solution of sucrose intravenously. Death occurred on the twenty-fourth day after operation and the first day after the second injection of sucrose.

Necropsy showed some precipitated albumin in the interstitial tissue in the area of the medulla of the kidney. Small collections of polymorphonuclear leukocytes were also present in the interstitial tissue. The epithelial cells of the tubules were greatly swollen, and their cytoplasm was foamy. The cytoplasm of some of these epithelial cells was homogeneous and stained deeply with eosin. The nuclei of many of the epithelial cells in the convoluted portion of the tubules were pyknotic, shrunken or absent. Some of the epithelial cells lining Bowman's spaces were swollen, and the cytoplasm was foamy. It was of interest to note that some of the epithelial cells in the convoluted portion of the tubules were apparently smaller than normal and stained more deeply with hematoxylin. These cells suggested those described in other cases as regenerative cells and did not show changes similar to those observed in the other tubules. Albumin, casts and occasional red and white blood cells were present in the lumens of the tubules. The glomeruli were frequently swollen.

The renal lesions were consistent with those that may occur after the injection of large quantities of sucrose.

CASE 16.—A white man aged 29 gave a history of chronic sinusitis with severe headache during the past month. After the patient's admission to the hospital signs of a brain abscess developed. At operation the abscess was drained. On the fifth day after operation the patient suddenly became comatose, the temperature rose to 39.3 C. (102.8 F.) and the respirations became rapid and shallow. An opening was made with a trephine over the midfrontal parietal region for the purpose of drainage. The patient died three hours after this operation.

On the day of death the patient was given 200 cc. of a 50 per cent solution of sucrose at 1:30 a. m. and a second injection of 200 cc. at 10:30 a. m. Death occurred at 2:20 p. m. He was given large amounts of dextrose solution in concentrations of 5 and 10 per cent for purposes of combating dehydration. Staphylococcus antitoxin was also given. He received one transfusion of 500 cc. of blood, without a reaction.

Essentially every epithelial cell lining the convoluted portion of the renal tubules was swollen, and the cytoplasm was foamy. Albuminous precipitate was present in the lumens of many of the tubules and in some of the spaces of Bowman.

The intake of fluid apparently was adequate. The patient was not appreciably dehydrated at any time. The renal lesions were similar to those that may occur after the injection of sucrose in experimental animals.

EXPERIMENTAL STUDY

Renal lesions have been produced in rabbits by Helmholz [11] and in dogs by Lindberg, Wald and Barker.[14] The quantity of sucrose administered and the length of time during which the animals were treated varied. Helmholz gave 1 rabbit 3,025 cc. of a 20 per cent solution of sucrose in twenty-one injections over a period of one hundred and eighty-two days. On the day after the last injection the blood contained 116 mg. of urea per hundred cubic centimeters and the excretion of phenolsulfonphthalein was only 5 per cent in two hours. The rabbit was weak and was killed. Focal nephritis was present. The author stated: "The protoplasm of the cells of the convoluted tubules was finely granular and the cells were swollen so as to occlude the lumens of a few of the tubules."

Lindberg and associates [14] gave 100 cc. of a 50 per cent solution of sucrose intravenously to dogs each day for as long as fourteen days. For 1 of these animals, treated for ten days, the microscopic diagnosis was as follows: "Few glomeruli showed retrogressive changes; some hemorrhages into glomerular tufts and changes; early degeneration of convoluted tubules." They described the kidneys from a second dog treated in the same way: "The tubules essentially normal; no marked changes seen in glomeruli." For the kidneys from a third dog given one injection of sucrose and killed twenty-four hours later the following description was given: "Moderate hyperemia of glomerular tuft; proximal and distal convoluted tubules showed intense swelling nearly obstructing tubules; glomeruli were swollen and hemorrhagic."

This wide variation in the susceptibility of dogs and rabbits to the intravenous injection of sucrose is very likely similar to the variations in the frequency of the occurrence of renal lesions in man following the intravenous injection of a hypertonic solution of sucrose. The clinical data suggest that the development of the tubular changes may be influenced by the state of hydration before and during the time sucrose is given. Apparently the factor of "hydration" has not been considered in the experimental production of renal lesions by the injection of sucrose. The experimental study reported here was made to determine the effect of restriction of fluid on the development of renal lesions when a hypertonic solution of sucrose is given intravenously.

Method and Material.—Normal rabbits, weighing 1.5 to 3.0 Kg. each, were used. A 50 per cent solution of sucrose [16] was injected into the marginal veins of the ear in various quantities and at different times and intervals. Blood for the determination of nonprotein nitrogen [17] was taken from the heart. Frequent observations were made on the animals' weight.

The rabbits were fed cabbages, oats and water. In those experiments in which the fluid was restricted only oats were fed.

The rabbits were killed at various intervals. The kidneys were removed and fixed immediately in a 10 per cent concentration of solution of formaldehyde U. S. P. A complete autopsy was performed on some of the animals. Material for sectioning was embedded in paraffin, and the sections were stained with hematoxylin and eosin.

EXPERIMENT 1.—A group of 8 rabbits were given various quantities of sucrose, as shown in table 1. Two animals in this group which died within the first twenty-four hours are not included in the table. The intervals during which fluid was restricted were only twenty-four to forty-eight hours. There is some indication, however, from these data that restriction of fluids may affect the retention of the nonprotein nitrogen.

Rabbit 97 on the day before death was drowsy and inactive. No muscular twitching or convulsions were observed. This animal refused water on the morning before his death in the afternoon. At autopsy the kidneys were swollen and pale. The epithelial cells in the convoluted tubules were swollen, and the lumens were essentially occluded as a result of this swelling. There was little abnormal to be seen in the cytoplasm of the epithelial cells. A few pink threads of fibrin-like material were present. The nuclei were pyknotic, with irregular peripheries. No changes were observed in the glomeruli. Precipitated albumin-like material was present in Bowman's space in some of the glomeruli.

EXPERIMENT 2.—In a second group, of 18 rabbits, cabbage and water were removed from the diet for a much longer time before and during the period the sucrose was given. Table 2 shows the intervals during which the rabbits were given only oats and the quantity of sucrose administered to each animal. This table also gives the amount of nonprotein nitrogen in the blood. Each of the 6 rabbits (116, 117, 118, 122, 123 and 124) given only oats for four days before

16. Eli Lilly & Co. supplied the sucrose used in these experiments.

17. Mrs. R. L. Wyatt, technician in the chemistry laboratory of the Division of Medicine, determined the nonprotein nitrogen in the blood.

TABLE 1.—*Retention of Nonprotein Nitrogen in Rabbits Given Sucrose* *

Duration of Experiment, Day	Rabbit					
	96	97	98	S-1	S-2	S-3
1	11:05 a.m. 15 cc. 4:00 p.m. 54 mg.	11:05 a.m. 15 cc. 4:00 p.m. 53 mg.	10:45 a.m. 34 Mg. 10:45 a.m. 15 cc. 1:30 p.m. 15 cc. 4:15 p.m. 34 mg.	9:00 a.m. 37 mg. 9:00 a.m. 20 cc. 11:15 a.m. 10 cc. 3:30 p.m. 20 cc.	9:00 a.m. 46 mg. 9:00 a.m. 20 cc. 11:15 a.m. 10 cc. 3:30 p.m. 20 cc.	9:00 a.m. 42 mg. 9:00 a.m. 20 cc. 11:15 a.m. 10 cc. 3:30 p.m. 20 cc.
2	.....	.....	9:30 a.m. 20 cc. 1:15 p.m. 20 cc. 3.45 p.m. 55 mg.	10:00 a.m. 45 mg.	10:00 a.m. 54 mg.	10:00 a.m. 55 mg.
3	.....	.....	.....	8:00 a.m. 30 mg.	8:00 a.m. 32 mg.	10:00 a.m. 55 mg.
4	10:30 a.m. 38 mg. 10:30 a.m. 15 cc.	10:30 a.m. 46 mg. 10:30 a.m. 15 cc.	.....	9:30 a.m. 20 cc. 10:45 a.m. 20 cc. 11:30 a.m. 20 cc.	9:30 a.m. 20 cc. 10:45 a.m. 20 cc. 11:30 a.m. 20 cc.	9:30 a.m. 20 cc. 10:45 a.m. 20 cc. 11:30 a.m. 20 cc.
5	9:30 a.m. 15 cc. 1:15 p.m. 20 cc. 3:35 p.m. 28 mg.	9:30 a.m. 20 cc. 1:15 p.m. 20 cc. 3:45 p.m. 29 mg.	9:00 a.m. 20 cc.† 11:30 a.m. 20 cc. 1:30 p.m. 20 cc. 4:00 p.m. 45 mg.	.....	.....	.....
6	.....	.....	10:00 a.m. 45 mg.	8:15 a.m. 47 mg.	8:15 a.m. 34 mg.	8:15 a.m. 39 mg.
7	.....	.....	.....	8:00 a.m. 53 mg.	8:00 a.m. 54 mg.	8:00 a.m. 50 mg.
8	9:00 a.m. 30 cc. 11:30 a.m. 30 cc. 1:30 p.m. 30 cc. 4:00 p.m. 52 mg. Killed	9:00 a.m. 20 cc.† 11:30 a.m. 20 cc. 1:30 p.m. 20 cc. 4:00 p.m. 200 mg.	9:00 a.m. 187 mg.	8:30 a.m. 20 cc. 10:00 a.m. 20 cc.	8:30 a.m. 20 cc.† 10:00 a.m. 20 cc.	8:30 a.m. 10 cc.† 10:00 a.m. 20 cc.
9	.....	10:00 a.m. 353 mg. 4:30 p.m. Died	9:00 a.m. 260 mg.	8:00 a.m. 64 mg.	8:00 a.m. 43 mg.	8:00 a.m. 37 mg.
10	.....	.....	9:00 a.m. 176 mg.	8:15 a.m. 54 mg.	8:00 a.m. 88 mg.	8:15 a.m. 28 mg.
11	.....	.....	9:00 a.m. 143 mg.	8:00 a.m. 45 mg.	8:00 a.m. 45 mg.	8:00 a.m. 27 mg.
12	.....	.....	9:00 a.m. 88 mg.	.....	.....	.....
13	.....	.....	9:00 a.m. 66 mg.	.....	8:00 a.m. 34 mg.	8:00 a.m. 28 mg.
14	.....	.....	.....	9:30 a.m. 20 cc.† 11:00 a.m. 20 cc. 1:30 p.m. 20 cc. 4:00 p.m. 32 cc.	9:30 a.m. 20 cc.† 11:00 a.m. 20 cc. 4:00 p.m. 36 mg.	4:00 p.m. 47 mg.†
15	.....	.....	9:00 a.m. 44 mg.	9:00 a.m. 51 mg.	9:00 a.m. 41 mg.	9:00 a.m. 47 mg.
16	.....	.....	9:00 a.m. 55 mg.	9:00 a.m. 31 mg.	9:00 a.m. 43 mg.	9:00 a.m. 28 mg.
17	.....	.....	8:00 a.m. Died			
22	.....	.....	.....	8:30 a.m. 60 cc.†	8:30 a.m. 60 cc.†	8:30 a.m. 60 cc.†
23	.....	.....	.....	8:30 a.m. 33 mg.†	8:30 a.m. 30 mg.†	8:30 a.m. 40 mg.†
24	.....	.....	.....	8:30 a.m. 33 mg.	8:30 a.m. 34 mg.	8:30 a.m. 26 mg.
28	.....	.....	.....	9:30 a.m. 20 cc.‡ 1:30 p.m. 20 cc. 4:00 p.m. 20 cc.	9:30 a.m. 20 cc.‡ 1:30 p.m. 20 cc. 4:00 p.m. 20 cc.	9:30 a.m. 20 cc.‡ 1:30 p.m. 20 cc. 4:00 p.m. 20 cc.
29	.....	.....	.....	8:00 a.m. 32 mg.	8:00 a.m. 38 mg.	8:00 a.m. 97 mg.
30	.....	.....	.....	8:00 a.m. 43 mg. Experiment discontinued	8:00 a.m. 38 mg. Experiment discontinued	8:00 a.m. 36 mg. Experiment discontinued

* All measurements in cubic centimeters refer to intravenous injection of a 50 per cent solution of sucrose, and all those in milligrams signify the amount of nonprotein nitrogen per hundred cubic centimeters of blood.
† No water for twenty four hours.
‡ No water for twenty four hours before and twenty four hours after the injection of sucrose.

TABLE 2.—*Effect of Restriction of Fluids on Retention of Nonprotein Nitrogen in Rabbits Given Sucrose* *

Diet	Rabbit	Duration of Experiment, Days									
		1	2	3	4	5	6	7	8	9	10
Only oats for 3 days before and during experiment	110†	3:30 p.m.‡ 55 mg.	.....	.....	.....	.....	.....	..	.....	.....	.....
	111	3:30 p.m.‡ 122 mg.	.....	.....	.....	.....	.....	..	.....	.....	.....
	112	3:30 p.m.‡ 43 mg.	.....	.....	.....	.....	.....	..	.....	.....	.....
Oats, water and cabbage for 3 days before and during experiment	113	3:30 p.m.‡ 43 mg.	.....	.....	.....	.....	.....	..	.....	.....	.....
	114	3:30 p.m.‡	.....	.....	.....	.....	.....	..	.....	.....	.....
	115	3:30 p.m.‡	.....	.....	.....	.....	.....	..	.....	.....	.....
Only oats for 4 days before and during experiment	116	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9 a.m. 20 cc. 5 p.m. 120 mg.	4:00 p.m. 120 mg.	.....	..	3:00 p.m. 316 mg.	5:00 p.m.‡ 429 mg.	.....
	117	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9 a.m. 20 cc. 5 p.m. 154 mg.	4:00 p.m. 65 mg.	.....	..	3:00 p.m.‡ 375 mg.	.....	.....
	118	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9 a.m. 20 cc. 5 p.m. 139 mg.	.....	.....	..	.....	.....	.....
Oats, cabbage and water for 4 days before and during experiment	119	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m.‡ 38 mg.	.....	.....	.....	..	.....	.....	.....
	120	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9:00 a.m. 20 cc.	4:00 p.m. 67 mg.	.....	..	3:00 p.m. 38 mg.	.....	9:00 a.m.‡ 30 mg.
	121	9:30 a.m. 20 cc.	9:00 a.m. 20 cc.	10:00 a.m. 20 cc.	9:00 a.m. 20 cc.	4:00 p.m. 75 mg.	.....	..	3:00 p.m. 40 mg.	.....	9:00 a.m.‡ 34 mg.
Only oats for 4 days before and during experiment	122	9:00 a.m. 36 mg. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 54 mg.‡ 9 a.m. 20 cc. 4 p.m. 50 mg.	.....	.....	.....	..	.....	.....	.....
	123	9:00 a.m. 50 mg. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 64 mg. 9 a.m. 20 cc. 4 p.m. 82 mg.	.....	9:00 a.m. 200 mg.	.....	..	4:00 p.m.‡ 214 mg.	.....	.....
	124	9:00 a.m. 34 mg. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 53 mg. 9 a.m. 20 cc. 4 p.m. 62 mg.	3:00 p.m. 20 cc.	9:00 a.m. 88 mg.	4:00 p.m.‡ 100 mg.	..	.....	.....	.....
Only oats for 4 days before experiment; cabbage added when sucrose was first injected	125	9:00 a.m. 54 mg. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 49 mg. 9 a.m. 20 cc. 4 p.m. 43 cc.	3:00 p.m. 20 cc.	9:00 a.m. 30 mg.	Experiment discontinued	..	.....	.....	.....
	126	9:00 a.m. 20 cc.	9:00 a.m. 20 cc.	9 a.m. 42 mg. 9 a.m. 20 cc. 4 p.m. 41 mg.	3:00 p.m. 20 cc.	9:00 a.m. 39 mg.	4.00 p.m. 36 mg.	..	.....	.....	.....
	127	.....	.....	9 a.m. 41 mg. 9 a.m. 20 cc. 4 p.m. 32 mg.	3:00 p.m. 20 cc.	9:00 a.m. 30 mg.	4:00 p.m.‡ 55 mg.	..	.....	.....	.....
Only oats for 4 days before and during experiment	128	9:00 a.m. 50 mg.	9:00 a.m. 51 mg.	.....	.....	9:00 a.m. 53 mg.	Experiment discontinued	..	.....	.....	.....
	129	.....	9:00 a.m. 67 mg.	.....	.....	9:00 a.m. 68 mg.	Experiment discontinued	..	.....	.....	.....

* All measurements in cubic centimeters refer to intravenous injection of a 50 per cent solution of sucrose, and all those in milligrams signify the amount of nonprotein nitrogen per hundred cubic centimeters of blood.

† Rabbits 110 to 115 were given 15 cc. of sucrose solution at 10 a.m. and at 1 p.m. and were killed at 3:30 p.m.

‡ Killed.

the sucrose was injected showed a retention of nonprotein nitrogen either after the third or after the fourth injection. The controls (rabbits 119, 120, 121, 125, 126 and 127) did not exhibit a corresponding retention of nonprotein nitrogen. In fact, there was only a slight retention of nonprotein nitrogen by rabbits 120 and 121, and in the others this factor was normal.

Two rabbits (128 and 129), as shown in table 2, were given only oats for four days, and the nonprotein nitrogen in their blood was determined on the fourth, the fifth and the seventh day. It was only slightly elevated.

The rabbits given only oats and sucrose rapidly lost weight. Those given oats, cabbage and water during the time sucrose was injected showed insignificant variations in weight. In fact, some of the animals gained. Rabbits given only

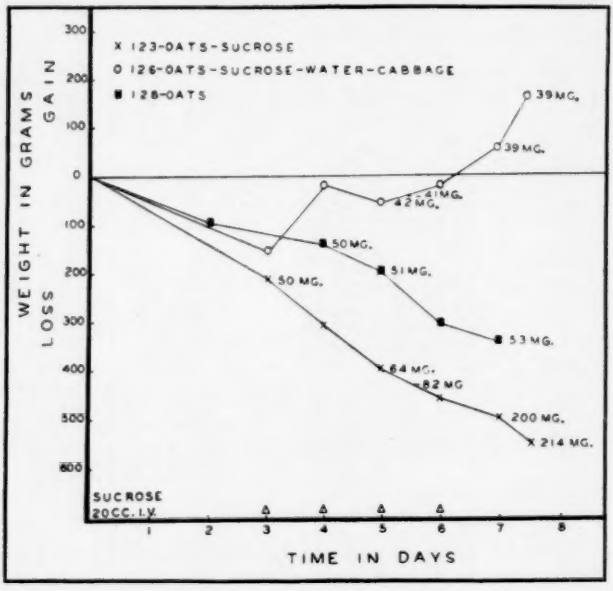

Fig. 1.—The effect of sucrose on the weight of rabbits and their retention of nonprotein nitrogen when the fluid intake is restricted.

oats lost weight; however, this was much less than when sucrose was also given. The variations in the weight of the different groups of rabbits are illustrated by the data in figure 1.

The kidneys in rabbits 110, 111, 112, 113, 114 and 115 showed essentially the same degree of tubular injury. The cells in the convoluted portion of the renal tubules were slightly swollen, and the cytoplasm was finely granular. The kidneys from rabbits 116, 117, 118, 122 and 123 showed the cells in the convoluted portion of the tubules to be greatly swollen, the cytoplasm vacuolated and the nuclei pyknotic. The lumens of the tubules were frequently occluded. Precipitated albuminous material was present in Bowman's space in some of the glomeruli and in the lumen in some of the tubules. No lesions were observed in the glomeruli, in Henle's loops or in the collecting tubules. The renal changes in rabbits 119,

Fig. 2.—*A* (rabbit 118), the lumens of tubules almost occluded by the greatly swollen epithelial cells. This rabbit was fed only oats for four days before and during the experiment. Twenty cubic centimeters of a 50 per cent solution of sucrose was injected intravenously each day for four days. Eight hours after the last injection the blood contained 139 mg. of nonprotein nitrogen per hundred cubic centimeters. The rabbit was killed on the fourth day of the experiment. *B* (rabbit 121), normal appearance of the kidneys. This rabbit was fed oats, cabbage and water for four days before and during the experiment. It was given the same amount of sucrose as was administered to rabbit 118. At the time rabbit 121 was killed, six days after the last injection of sucrose, the blood contained 34 mg. of nonprotein nitrogen per hundred cubic centimeters.

126 and 127 were similar to those in rabbit 110. It would appear that the lesion in the epithelial cells of the tubules in this group of rabbits varies only in degree from that in the rabbits given sucrose and fed oats, water and cabbage.

The degree of the pathologic change in the kidney of rabbit 124 appears to be about midway between that of rabbit 127 and rabbit 123. The nonprotein nitrogen value for rabbit 124 was 100 mg. per hundred cubic centimeters of blood, while that for rabbit 123 was 214 mg. and that for rabbit 127 was 55 mg.

There were no pathologic changes in the epithelial cells of the convoluted portion of the renal tubules in rabbits 120 and 121. The nonprotein nitrogen was also normal. It is important to observe in table 2 that the nonprotein nitrogen values for these 2 animals were 67 and 75 mg., respectively, per hundred cubic centimeters on the fifth day of the experiment. On the tenth day, when the animals were killed, the values were normal.

There were no pathologic changes in the other viscera of these animals that we can at present associate with the administration of sucrose.

EXPERIMENT 3.—Of a group of 5 rabbits, 2 were fed oats, cabbage and water and 3 were fed only oats. On the fourth day of the experiment each rabbit was given 25 cc. of a 50 per cent solution of sucrose. This injection of sucrose was repeated on the four following days. On the day following the last injection of sucrose the nonprotein nitrogen in the blood of the 2 rabbits given cabbages, oats and water was within the range of normal, while each of the other animals showed a retention of nonprotein nitrogen in the blood.

COMMENT

The quantity of sucrose given to the patients in cases 1, 3 and 7 was much less than that given to patients by Dyar and Matthew [7] and by Masserman.[8] Renal lesions were present in each of our patients. The investigators just mentioned failed to observe any "unfavorable" reactions in their patients, although a larger quantity of sucrose was injected by the same route. Anderson [13] concluded from his studies that previous renal lesions influenced the development of tubular lesions following the injection of sucrose. Murphy and his associates [2] gave 300 to 500 cc. of a 50 per cent solution of sucrose to a group of 21 patients with chronic hypertension and observed improvement, without unfavorable results. It appears that previous renal disease, such as glomerular and arteriolosclerotic nephritis, has little if any effect on the development of renal lesions following the injection of sucrose.

An insufficient amount of fluid apparently was given to some of our patients in whom tubular lesions developed after the injection of sucrose. In case 1 the patient was unconscious for approximately forty-eight hours before he was admitted to the hospital. He was given 200 cc. of a 25 per cent solution of sucrose one hour after admission and died three hours later. This case illustrates the rapidity with which this tubular lesion may develop. This has been discussed by Anderson.[13] The quantity of fluid given in case 10 after the third day apparently was adequate. The fluid output was low. It would seem that the epithelial

cells lining the convoluted tubules of the kidney may have become swollen on the second day after the injection of sucrose. The lumens of the tubules were blocked as a result of the swelling of these cells. Helmholz [11] discussed the occlusion of the lumen of the tubules in rabbits given sucrose.

Our experimental observations on the rabbit show that in animals given sucrose but no fluid renal lesions develop and nonprotein nitrogen is retained in the blood. The quantity of sucrose necessary to produce renal lesions in dehydrated rabbits is much less than that used by Helmholz [11] to produce renal lesions.

There are insufficient clinical data to indicate the effect of this renal lesion on renal function. The experimental results of Helmholz [11] and Lindberg and associates,[14] as well as ours, show that the renal function is definitely diminished after the injection of sucrose.

A knowledge of the quantity of sucrose that will produce renal lesions is important. In cases 4 and 5 the patients were given 100 cc. each of a 25 per cent solution of sucrose intravenously. No renal lesions were present. It is difficult to determine the degree of hydration of these patients at the time sucrose was given. The patient in case 11 was a child 8 years old. She was given adequate fluids during the time she received the sucrose. There was no retention of nonprotein nitrogen. In case 12 the patient was given adequate fluids and two intravenous injections of 100 cc. each of a 25 per cent solution of sucrose. No retention of nonprotein nitrogen occurred. The pathologic studies on rabbits suggest that there is a definite correlation between the development of tubular lesions and the retention of nonprotein nitrogen in the blood.

Multiple injections of sucrose were given to a group of patients by Hilton and Alderson.[8] The total quantity, however, was much less than that given to several of the patients included in the present series. It seems unlikely that the occurrence of these renal lesions is directly influenced by a previous injection of sucrose. The chemical and pharmacologic action of sucrose has been discussed by Keith and Power,[9] Keith [18] and Bullock, Gregersen and Kinney.[19]

The pathologic lesions observed in our experimental animals and in the patients appear to be identical and to be similar to those reported by other investigators. We did not observe glomerular lesions like those described by Lindberg and his associates.[14] The results of the histologic studies and the observations on the retention of nonprotein nitrogen suggest a correlation between these pathologic changes and renal func-

18. Keith, N. M.: Experimental Dehydration, Am. J. Physiol. **68**:80, 1924.

19. Bullock, L. T.; Gregersen, M. I., and Kinney, R.: The Use of Hypertonic Sucrose Solution Intravenously to Reduce Cerebrospinal Fluid Pressure Without a Secondary Rise, Am. J. Physiol. **112**:82, 1935.

tion. It is suggested from the studies on rabbits 120, 121, 126 and 127 that the epithelial changes in the tubules may be transient and that the quantity of sucrose is important in the production of the renal lesions.

This renal lesion was diffuse in all of the rabbits and in a majority of the patients. In some of the patients with arteriolosclerotic nephritis the epithelial cells lining the tubules were smaller than normal in focal areas of the kidney. Dr. R. A. Moore and Dr. W. D. Forbus discussed this factor when Anderson [19] presented his paper at a meeting of the Southern Medical Association. The epithelial cells lining the convoluted portion of the tubules in those cases in which the changes are focal are frequently much lower and stain more deeply with hematoxylin than do corresponding cells in normal kidneys. The appearance of these epithelial cells and their failure to respond to sucrose as do other tubular epithelial cells may be similar to the effect of uranium nitrate on previously injured tubular epithelial cells, observed by MacNider.[20]

SUMMARY

A group of 16 clinical cases in which a hypertonic solution of sucrose was given intravenously is reported. In some instances the epithelial cells in the convoluted portion of the renal tubules were swollen. From a review of these cases it appears that dehydration is one of the most important factors in determining whether or not this tubular lesion will occur after the intravenous injection of sucrose. The quantity of sucrose administered during a given interval is also important.

Renal lesions similar to those in man may be produced experimentally by giving rabbits sucrose intravenously. The quantity of sucrose necessary to produce these lesions is much less when the fluid is restricted before and during the experiment. In fact, when fluids are given it is difficult to produce renal lesions in rabbits with sucrose unless large quantities are used.

20. MacNider, W. de B.: Urine Formation During the Acute and Chronic Nephritis Induced by Uranium Nitrate, in Harvey Lectures, 1928-1929, Baltimore, Williams & Wilkins Company, 1930, p. 82.

Progress in Internal Medicine

LIVER AND BILIARY TRACT

A REVIEW FOR 1941

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FUNCTIONAL ACTIVITY OF THE COMMON BILE DUCT AND THE SPHINCTER OF ODDI

The physiology of the biliary tract was reviewed in 1937.[1] At that time the various factors chiefly affecting the flow of bile with especial reference to the secretory pressure of the liver, the contractility and storage function of the gallbladder, the resistance of the sphincter of Oddi and the effect of changes in duodenal tone and motility were discussed in some detail. The literature dealing with the sphincter mechanism of the common bile duct in human subjects has been comprehensively reviewed by Bergh.[1a] The recognition that the flow of bile through the various portions of the biliary tract is subject to physiologic control has had broad clinical implications, and an extensive clinical literature dealing with disturbances in this functional control has arisen in consequence. One of the most recent experimental analyses of the mechanics of the bile flow has been that of Necheles and Kozoll.[2] They took separate records of the contractions of the gallbladder and of the duodenum, of the tone of the sphincter of Oddi, of respiration and of the blood pressure. They found that the sphincter of Oddi in dogs possesses a regular and distinct tonus rhythm. This was entirely independent of the tonus of the gallbladder, and there was no definite

From the Clinic for the Study of Disease of the Liver and Biliary Tract of the Department of Medicine and the Department of Surgery, New York Post-Graduate Medical School and Hospital, Columbia University.

1. Greene, C. H.; Handelsman, M. B., and Babey, S. M.: Liver and Biliary Tract: A Review for 1936, Arch. Int. Med. **59**:724 (April) 1937.

1a. Bergh, G. S.: The Sphincter Mechanism of the Common Bile Duct in Human Subjects: Its Reaction to Certain Types of Stimulation, Surgery **11**:299 (Feb.) 1942.

2. Necheles, H., and Kozoll, D. D.: A Study of the Sphincter of Oddi in the Human and in the Dog, Am. J. Digest. Dis. **9**:36 (Jan.) 1942. Kozoll, D. D., and Necheles, H.: A Study of the Mechanics of Bile Flow: I. Responses to Physiological Intravenous Solutions, Surg., Gynec. & Obst. **74**:27 (Jan.) 1942; II. Response to Intraduodenal Solutions, ibid. **74**:692 (March) 1942; III. Responses to Pharmacological Stimuli, ibid., to be published.

correlation between the activity of the duodenum and that of the sphincter of Oddi. In many cases the sphincter of Oddi and the duodenal musculature showed the same type of response to stimulation or to drugs, but the responses were independent of each other.

Kozoll and Necheles report that the intravenous injection of physiologic or hypertonic solutions of sodium chloride induces spasm both of the sphincter and of the duodenum. Dextrose solutions, on the other hand, produced a relaxation of the duodenum, with slight or only moderate increase in the tone of the sphincter. Their studies were extended to include a group of patients with a T tube in the common bile duct. An extensive pharmacologic study showed that the response to drugs of the epinephrine group, i. e., epinephrine, ephedrine, epinine (dihydroxyphenylethylmethylamine), propadrine hydrochloride and neosynephrin hydrochloride, was inconstant. Atropine reduced the sphincter tone or the response of the sphincter to saline solutions in the dog. When the drug was given to patients in doses sufficient to produce discomfort there was some increase in the resistance of the sphincter. The response to papaverine was inconstant. Morphine and codeine produced spasm of the sphincter. Glyceryl trinitrate, amyl nitrite and the hydrochloride of diphenylacetyldiethylaminoethanol (trasentin) all reduced the tone of the sphincter and were able to cause relaxation after the administration of codeine. Prostigmine usually increased both the tone of the sphincter and the motility of the duodenum. Necheles and Kozoll and Bergh [3] found that a fatty meal of egg yolk and cream often caused a slight initial contraction of the sphincter. This was then followed by a relaxation for sometimes as long as two to three hours after the meal. Bergh found that olive oil was less effective in reducing the tone of the sphincter than were egg yolk and cream. Protein and carbohydrate meals were without significant effect on the tone of the sphincter.

Mirizzi was one of the pioneers in the development of the technic of cholangiography. He [4] has recently presented a summary of his experience with cholangiography and his views regarding functional disturbances of the sphincter of Oddi. He reports cholangiograms which he interprets as demonstrating spasm and the presence of peristaltic waves in the common duct. MacDonald [5] also reports a case in which he found cholangiographic evidence of peristalsis in the terminal portion of the common duct and the papilla of Vater. Carter and Maraffino [6] have observed a case in which a similar process was evident.

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3. Bergh, G. S.: The Effect of Food upon the Sphincter of Oddi in Human Subjects, Am. J. Digest. Dis. **9**:40 (Jan.) 1942.

4. Mirizzi, P. L.: Functional Disturbances of the Choledochus and Hepatic Bile Ducts, Surg., Gynec. & Obst. **74**:306 (Feb., no. 2A) 1942.

5. MacDonald, D.: Common Bile Duct Peristalsis: Preliminary Report, Surg., Gynec. & Obst. **73**:864 (Dec.) 1941.

6. Carter, R. F., and Maraffino, B.: Personal communication to the author.

Mirizzi stresses the frequency of functional spasm of the sphincter of Oddi and insists that such spasm is associated with hypertrophy of the sphincter that still further interferes with the free passage of bile. He also considers the reflux of contrast medium into the duct of Wirsung a characteristic pathologic finding in the presence of dystonia of the sphincter of Oddi. Such reflux into the pancreatic ducts he believes to be a frequent cause of anatomic changes in the pancreas. This view is not very different from those usually held in this country, though most authors would not be willing to admit that pancreatic reflux is necessarily evidence of spasm of the sphincter of Oddi.

ETIOLOGY OF GALLSTONES

The various theories regarding the etiology of stones in the gallbladder were reviewed in detail by Phemister and his associates [7] and by Carter, Greene, Twiss and Hotz.[8] The last-named authors pointed out that no single mechanism would explain the formation of all the various types of gallstones. Their experience emphasized the relative importance of biliary stasis as compared to infection. A new and original point of view has recently been presented by Martensson.[9] He noted that many gallstones contained a central nucleus of necrotic epithelial cells and that sporulating bacilli were found in the epithelial cell walls in some cases of chronic cholecystitis. Cultures of material from diseased gallbladders removed at operation or at necropsy commonly showed the presence of what the author described as a typical bacillus. This was a gram-positive, motile, endosporulating rod. Different strains varied, some being facultative, others being obligatory, anaerobes. The bacillus did not fit any previously described organism in all its characteristics. Martensson was able to produce gallstones experimentally in rabbits, pigs, sheep and cattle by intravesicle, intravenous, subcutaneous or oral administration of cultures of this organism. If rabbits were first immunized against the organism, then biliary calculi did not develop after the injection of cultures.

It has long been recognized that organisms of the type of Bacillus welchii or Bacillus proteus vulgaris can be grown with a fair degree of regularity from cultures of the liver, bile or wall of the gallbladder.

7. Phemister, D. B.; Aronsohn, H. G., and Pepinsky, R.: Variations in the Cholesterol, Bile Pigment and Calcium Salts Contents of Gall Stones Formed in the Gall Bladder and in Bile Ducts with the Degree of Associated Obstruction, Ann. Surg. **109**:161 (Feb.) 1939.

8. Carter, R. F.; Greene, C. H.; Twiss, J. R., and Hotz, R.: Etiology of Gallstones: Critical Survey of the Literature and a Study of the Applicability of Various Theories in Two Hundred and Thirty-Nine Operative Cases, Arch. Surg. **39**:691 (Nov.) 1939.

9. Martensson, K.: Studies on the Etiology of Gallstones: A Subtilis-Like Bacilli-Group as an Etiologic Factor, Acta chir. Scandinav. **84**:227, 1941.

The culture and identification of the various anaerobic organisms require a careful and painstaking technic and so are not often done. The relation between this organism and the other anaerobic organisms described is still indeterminate. Without independent verification and further study this work cannot be accepted as conclusive, but it is most interesting and should stimulate further investigation.

GIARDIASIS AND THE BILIARY TRACT

Giardia lamblia is not an uncommon intestinal flagellate and often has been recognized in duodenal contents after drainage. It usually has been considered a harmless organism, and little attention has been paid to its presence. That it does enter the gallbladder is shown by the reports of a few cases [10] in which parasites were reported in bile obtained by aspiration from the gallbladder at operation or necropsy. The clinical significance of such infestation was undecided for a long time because of the absence of any effective method of treatment. The introduction by Galli-Valerio of atabrine in 1937 [11] as a specific remedy for giardiasis has been amply confirmed by the reports of de Muro,[12] Morrison and Swalm,[13] Nutter, Rodaniche and Palmer [14] and Hartman and Kyser.[15] This treatment is effective in causing the disappearance of the flagellate both from the stool and from the material obtained by duodenal drainage. Many patients with giardiasis complain of moderate or intermittent diarrhea as well as mild digestive disturbances. After the eradication of the parasite the symptoms often disappear. These results therefore indicate that giardias are mildly pathogenic for man. Their significance in regard to the biliary tract per se is still undetermined, but in view of the effectiveness of atabrine therapy, this question is of minor significance.

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10. Westphal, K., and Georgi: Ueber die Beziehungen der Lamblia intestinalis zu Erkrankungen der Gallenwege und Leber, München. med. Wchnschr. **70**:1080 (Aug. 17) 1923. Smithies, F.: Parasitosis of the Bile Passages and Gallbladder: A Report on Thirty-Seven Instances of Protozoiasis and One Instance of Infestation by Necator Americanus, Am. J. M. Sc. **176**:225 (Aug.) 1928. Calder, R. M., and Rigdon, R. H.: Giardia Infestation of Gallbladder and Intestinal Tract, ibid. **190**:82 (July) 1935. Hartman, H. R.; Kyser, F. A., and Comfort, M. W.: Infection of the Gallbladder by Giardia Lamblia, J. A. M. A. **118**:608 (Feb. 21) 1942.

11. Galli-Valerio, B.: La lambliase et son traitement par l'atébrine, Schweiz. med. Wchnschr. **67**:1181 (Dec. 11) 1937.

12. de Muro, P.: Atebrinbehandlung bei Giardiasis (Lambliasis), Deutsche med. Wchnschr. **65**:262 (Feb. 17) 1939.

13. Morrison, L. M., and Swalm, W. A.: A New Effective Parasiticide in Giardiasis, Am. J. Digest. Dis. **6**:325 (July) 1939.

14. Nutter, P. B.; Rodaniche, E. C., and Palmer, W. L.: Giardia Lamblia Infection in Man, J. A. M. A. **116**:1631 (April 12) 1941.

15. Hartman, H. R., and Kyser, F. A.: Giardiasis and Its Treatment: A Clinical Study, J. A. M. A. **116**:2835 (June 28) 1941.

CIRCULATION OF THE LIVER

The circulation of the liver is unique in that this organ receives both an arterial and a venous supply of blood. Their relative importance is still undetermined, though a subject of intensive study. Grindley, Herrick and Mann [16] measured the flow of blood to and from the liver in unanesthetized dogs. From these data Snyder [17] calculates that the blood flow in the hepatic artery is approximately one seventh of that in the portal vein.

The importance of the blood flow in the portal vein was indicated by the earlier observation that regeneration of the liver was not observed in animals with Eck fistulas. Higgins, Mann and Priestley [18] likewise found that regeneration after removal of a portion of the liver was not as active in birds as it was in mammals in which the whole of the portal blood flows through the liver. In his most recent experiments Mann [19] reports further evidence that the restoration of hepatic tissue after extirpation or injury depends almost completely on the supply of portal blood. The results of Grindley, Herrick and Mann showed considerable difference between the flow of blood to and from the liver, thus confirming older clinical impressions indicating the importance of the liver as a site for the storage of blood.

Numerous anatomic studies,[20] especially those of Pfuhl, Tischendorf, Arey and associates and Tyler, have served to establish the existence of mechanisms capable of distributing and regulating the flow of blood through the liver. In the hepatic veins and venules of the dog the smooth muscle cells are not evenly arranged in a continuous sheet as in ordinary veins but are grouped in spiral bands. At the opening of the hepatic venules into the hepatic veins, these bands are thickened and annular so as to provide sphincters. Similar annular sphincters are present at the openings of the hepatic veins into the inferior vena cava. Deysach [21]

16. Grindley, J. H.; Herrick, J. F., and Mann, F. C.: Measurement of the Blood Flow of the Liver, Am. J. Physiol. **132**:489 (March) 1941.

17. Snyder, C. D.: Recent Advances in Knowledge of the Liver, Physiol. Rev. **22**:54 (Jan.) 1942.

18. Higgins, G. M.; Mann, F. C., and Priestley, J. T.: Experimental Pathology of the Liver: X. Restoration of the Liver of the Domestic Fowl, Arch. Path. **14**:491 (Oct.) 1932.

19. Mann, F. C.: The Portal Circulation and Restoration of the Liver After Partial Removal, Surgery **8**:225 (Aug.) 1940.

20. Pfuhl, W.: Die Leber, in von Möllendorff, W.: Handbuch der mikroscopischen Anatomie des Menschen, Berlin, Julius Springer, 1932, vol. 5, pt. 2, p. 235. Tischendorf, F.: Histologische Beiträge zur Kenntnis der venosen Lebersperre, Ztschr. f. mikr.-anat. Forsch. **45**:266, 1939. Tyler, F. H.: A Note on the Microstructure of the Turtle's Liver, Anat. Rec. **79**:541 (April 25) 1941. Arey, L. B.; Sohlberg, R. J., and Rutherford, R. B.: Throttling Veins in the Livers of Certain Mammals, ibid. **81**:21 (Sept. 25) 1941.

21. Deysach, L. J.: Nature and Location of "Sphincter Mechanism" in Liver as Determined by Drug Actions and Vascular Injections, Am. J. Physiol. **132**:713 (April) 1941.

describes small endothelial tubes situated in the walls of the sublobular and central veins and connected with the veins through ostiums guarded by sphincters. Filling and emptying of these tubes by the action of the sphincters would provide a means of removing or adding considerable amounts of blood to the general circulation. The portal vessels differ from the hepatic venules in that they are devoid of smooth muscle.

These anatomic studies provide a mechanism for the regulation of the blood flow through the liver. Snyder and Tyler [22] summarize much of the evidence dealing with the different factors which affect that flow. Responses both to cholinergic, or vagal, and to adrenergic, or sympathetic, simulating agents are obtained but apparently are variable. These authors point out the numerous difficulties in the interpretation of results in studies of the hepatic blood flow, especially when a simultaneous study of the effect on the metabolic exchanges taking place in the liver is desired. Among other factors they emphasize the large amount of lymph formed in the liver and the effect of the resultant difference between the hepatic inflow and outflow.

Another method of observing certain phases of the hepatic circulation directly is reported by Wakim.[23] Using a method of transillumination, he was able to watch the circulation in the sinusoids and capillaries in frogs and rats. The circulation in the sinusoids was intermittent, with phases of activity alternating with periods of inactivity. During rest the sinusoids may be empty or may be packed with blood cells and apparently serve as storehouses. The hepatic artery was found to supply blood to the parenchyma through several routes. Some of the arterial terminals empty directly into the sinusoids. Other arterial branches empty into the portal vein proximal to the sinusoids. Direct arteriovenous anastomoses between the branches of the hepatic artery and the portal vein were also observed. It is possible that further work along this line will help increase the present day understanding of the intermittent or cyclic functions of the liver and the specific localization of the effects of some hepatic toxins.

Preliminary experiments on the latter problem are reported by Wakim and Mann.[24] They found that the inhalation of carbon tetrachloride produces an immediate vasoconstrictor effect on the circulation

22. Snyder, C. D.: Rev. Gastroenterol., to be published. Snyder, C. D., and Tyler, F. H.: Metabolism Studies on Surviving Turtle's Liver, J. Cell. & Comp. Physiol. **16**:377, 1940.

23. Wakim, K. G.: The Intrahepatic Circulation of Blood in the Intact Animal: Preliminary Report, Proc. Staff Meet., Mayo Clin. **16**:198 (March 26) 1941.

24. Wakim, K. G., and Mann, F. C.: Effect of Experimental Cirrhosis on the Intrahepatic Circulation of Blood in the Intact Animal, Arch. Path. **33**:198 (Feb.) 1942.

in the hepatic sinusoids in rats. If the carbon tetrachloride was removed at once, the circulation returned to its normal state within half a minute. If the inhalation of carbon tetrachloride was continued for thirty minutes, hepatic injury resulted. Maximal toxic effects were evident twenty-four hours later. At that time hepatic enlargement with fatty degeneration, hydropic changes and small hemorrhages were observed. Many of the sinusoids were obliterated from pressure of the swollen hepatic cells, and the liver was anemic in consequence. The sinusoids that remained active were tortuous and irregular in caliber from such pressure. Sinusoids supplied by the hepatic artery were more numerous than those arising from the portal vein. Several weeks after a single inhalation of carbon tetrachloride the livers had regained their normal appearance.

The repeated inhalation of carbon tetrachloride produced cirrhosis of the liver. Wakim and Mann found that in rats the vascular changes consisted of an early initial vasoconstriction during the period of inhalation. This was followed by a prolonged secondary obliteration of the vascular channels, especially venous, in consequence of the swelling and fatty degeneration of the hepatic parenchyma. With advanced cirrhosis there were further collapse and obliteration of the vessels. In the areas of regenerating hepatic parenchyma there was an extreme disorganization in the distribution of the blood vessels. The majority of the sinusoids in such areas were supplied by arterial rather than by venous blood. These studies, therefore, further emphasize the great disorganization of the blood supply in cirrhosis of the liver.

PORTAL HYPERTENSION AND THE CRUVEILHIER-BAUMGARTEN SYNDROME

The syndrome of portal hypertension is well recognized.[25] While in the majority of cases the syndrome is associated with hepatic cirrhosis, this is not necessarily so, and the clinical syndrome may be produced by any condition which produces portal hypertension. Such an occurrence is illustrated by a case reported by Reich,[26] in which portal phlebosclerosis was associated with apparently congenital narrowing of the portal vein.

A related type of disturbance is represented in the cases described as instances of the Cruveilhier-Baumgarten syndrome. The 55 cases of this syndrome on record have been reviewed by Armstrong and his

25. Greene, C. H.; Plotz, M., and Localio, S. A.: Liver and Biliary Tract: A Review for 1937, Arch. Int. Med. **61**:655 (April) 1938.

26. Reich, N. E.: Primary Portal Phlebosclerosis, Arch. Int. Med. **69**:117 (Jan.) 1942.

associates.[27] They point out that this title may be applied to the condition of patients having the clinical picture of portal hypertension featured by evidence of an excessive umbilical circulation and the presence of a loud abdominal murmur and thrill. The other symptoms, abdominal distention, digestive disturbances, hematemesis and splenomegaly, are to be related to the portal hypertension.

Armstrong and his associates divide these cases into several groups.

1 A.—Cases in which the clinical picture is associated with patency of the umbilical vein, atrophy of the liver with little or no cirrhosis and splenomegaly. This group represents a separate category dependent on the presence of a distinct congenital anomaly, namely, a patent umbilical vein. Armstrong and co-workers report these cases, 5 in all, as examples of Cruveilhier-Baumgarten disease. The atrophy of the liver they consider to be associated with hypoplasia of the portal system and reduction in the hepatic circulation.

1 B.—Cases in which the umbilical vein is patent but in which there is associated advanced portal cirrhosis. In this group the authors assume that the cirrhosis developed in a patient who coincidentally had a congenitally patent umbilical vein. The portal hypertension developing in consequence of the cirrhosis then led to further dilatation of the umbilical vein.

2 A.—Cases of portal hypertension from disease of the liver or portal system with unusually prominent paraumbilical veins. This is the largest group, and the situation is the most probable in cases of cirrhosis with loud venous hum or murmur or marked caput medusae.

2 B.—Cases of portal hypertension with patency of other collateral veins in the umbilical area.

This distinction between Cruveilhier-Baumgarten disease and Cruveilhier-Baumgarten syndrome and the reclassification of cases of the latter according to the vessels involved promises to obviate considerable confusion with regard to the recognition of these conditions in the future.

LEPTOSPIROSIS ICTEROHAEMORRHAGICA

The literature dealing with leptospirosis icterohaemorrhagica (Weil's disease) was reviewed by Greene and Farrell in 1940.[28] In the past year additional cases were reported from this country by Ashe, Pratt-

27. Armstrong, E. L.; Adams, W. L., Jr.; Tragerman, L. J., and Townsend, E. W.: The Cruveilhier-Baumgarten Syndrome: Review of the Literature and Report of Two Additional Cases, Ann. Int. Med. **16**:113 (Jan.) 1942.

28. Greene, C. H., and Farrell, E.: Liver and Biliary Tract: A Review for 1939, Arch. Int. Med. **65**:847 (April) 1940.

Thomas and Kumpe,[29] White and Prevost,[30] Rathbun and Waghelstein,[31] Reid and Holt[32] and Stiles and Sawyer.[33] The last-named authors report that they were able to collect a total of 73 authentic cases from North America, with half as many additional cases which had been reported but in which the laboratory proof of leptospirosis was not regarded as adequate. In a footnote they report their knowledge of more than a score of additional and unrecorded cases which were not included in their tabulations. These figures emphasize both the increasing importance of Weil's disease in the United States and the increasing frequency with which this clinical syndrome is being recognized by members of the medical profession.

Ashe and his associates divide the clinical course of the disease into three stages, which are important for diagnosis, though the line of clinical demarcation is not sharp. The onset is abrupt, with severe headaches, muscular pains, backache and high temperature. Marked leukocytosis usually is present. This is the so-called septicemic stage, with leptospiras circulating in the blood stream. They may be recognized in the serum by dark field examination. Stiles and Sawyer, as well as Fennel[34] and Raven,[35] cite an extensive literature, pointing out that confusing artefacts are commonly found on dark field examination of body tissues or fluids and that these pseudospirochetes have been reported in the tissues and fluids of persons in good health and in those of patients suffering from other diseases. The finding of leptospiras in the serum or urine on dark field examination, therefore, should always be confirmed by inoculation of a guinea pig.

The second stage of the disease is associated with the development of jaundice in 40 to 100 per cent of cases. The jaundice appears about the fifth day. At about this time the organisms disappear from the blood and appear in the urine, while specific antibodies appear in the serum.

29. Ashe, W. F.; Pratt-Thomas, H. R., and Kumpe, C. W.: Weil's Disease: A Complete Review of American Literature and an Abstract of the World Literature; Seven Case Reports, Medicine **20**:145 (May) 1941.

30. White, J. J., and Prevost, J. V.: Weil's Disease: Report of Three Cases, Including the Morbid Anatomy of One Case, and a Brief Review of the Pertinent Literature, Ann. Int. Med. **15**:207 (Aug.) 1941.

31. Rathbun, H. K., and Waghelstein, J. M.: Weil's Disease: Report of Six Cases, Ann. Int. Med. **15**:395 (Sept.) 1941.

32. Reid, J. D., and Holt, R. A.: Spirochetal Jaundice in Virginia with Special Reference to Laboratory Diagnostic Methods, Virginia M. Monthly **68**:571 (Oct.) 1941.

33. Stiles, W. W., and Sawyer, W. A.: Leptospiral Infection (Weil's Disease) as an Occupational Hazard, J. A. M. A. **118**:34 (Jan. 3) 1942.

34. Fennel, E. A.: Weil's Disease and Artefacts, Proc. Staff Meet., Clin., Honolulu, March 1938, vol. 4.

35. Raven, C.: Canine Leptospirosis in Pennsylvania, J. Infect. Dis. **69**:131 (Sept.-Oct.) 1941.

The third stage, or convalescence, begins in the latter part of the second week of the disease (fourteen to sixteen days after onset). By then defervescence occurs, and the jaundice begins to clear. A short relapse is not uncommon but usually is not serious. In the third and subsequent weeks of the disease specific agglutinins appear in the serum in high titer. Agglutinations in a dilution of 1 : 300 or over are generally considered as diagnostic. Likewise, a negative reaction after the thirtieth day of illness rules out Weil's disease.

The mortality increases with age. Stiles and Sawyer report a mortality in the American series of 17 per cent for patients under 40 years and of 63 per cent for patients over 40 years of age. The total mortality was 33 per cent. By contrast, the mortality in the Netherlands series was only 11.9 per cent. This difference, however, is probably due not to a difference in the severity of the infection but to the greater number of cases in the European series in which the disease was mild and jaundice was absent.

Stiles and Sawyer point out that if an occupation exposes a worker to contact with rats or dogs or to water or moist material contaminated by the urine of these animals, he is exposed to a risk of leptospiral infection greater than the risk run in private life. They therefore emphasize that under such circumstances Weil's disease is not an occupational disease but rather an occupational hazard. This is sufficiently accepted that Weil's disease is now a compensable disability under the workmen's compensation acts of England, Germany and Australia, as well as that of New York.

VITAMIN K AND BLOOD COAGULATION

Scientific interest in the problem of blood coagulation continues unabated. Howell [36] was one of the pioneers in the development of this field, so that it is most appropriate at the present time that he should summarize the recent advances. These are twofold. On the one hand, there is the introduction of heparin into clinical medicine as an anticoagulant to prevent the formation of thrombi after mechanical or chemical injury to the lining of the heart or blood vessels; on the other hand, there is the use of vitamin K to restore the coagulability of the blood to normal in cases of hypoprothrombinemia. The uses of heparin are not within the scope of this review.

The literature on the clinical and experimental studies on vitamin K has previously been reported in detail.[37] Reference was made then

36. Howell, W. H.: Recent Advances in the Problem of Blood Coagulation Applicable to Medicine, J. A. M. A. **117**:1059 (Sept. 27) 1941.

37. (*a*) Greene, C. H., and Hotz, R.: Liver and Biliary Tract: A Review for 1938, Arch. Int. Med. **63**:778 (April) 1939. (*b*) Greene, C. H.: Liver and Biliary Tract: A Review for 1940, ibid. **67**:867 (April) 1941.

to the numerous monographs on vitamin K which had appeared within the past year or so. This list has now been extended in the monographs of Butt and Snell [38] and Koller.[39]

Increased experience in the study of the prothrombin content of the blood in various conditions has led to general agreement that the prothrombin of the blood of newborn infants is decreased temporarily during the first week of life. This hypoprothrombinemia is an important factor in the causation of neonatal cerebral hemorrhage. Hypoprothrombinemia in the newborn can be corrected by injection of synthetic vitamin K [40] or by inunction with an ointment containing the vitamin,[41] but the simplest method is to give synthetic vitamin K to mothers, either orally or by injection during labor.[42] A satisfactory therapeutic response has

38. Butt, H. R., and Snell, A. M.: Vitamin K, Philadelphia, W. B. Saunders Company, 1941.

39. Koller, F.: Das Vitamin K und seine klinische Bedeutung, Leipzig, Georg Thieme, 1941.

40. Damm, P. N.: Hemorrhagic Diathesis in the New Born, Especially with Regard to Treatment with Blood Transfusion, Ugesk. f. læger **102**:620 (June 13) 1940. Fiechter, N.: Hypoprothrombinemia and Hemorrhagic Diathesis of the New Born and Its Relations to Vitamin K, Monatschr. f. Geburtsh. u. Gynäk. **111**:1 (Aug.) 1940. Grossman, A. M.: Coagulation Defects in Infancy and Childhood: Frequency of Hypoprothrombinemic States and Their Treatment with Vitamin K; Reclassification of Hemorrhagic Hypoprothrombinemia Neonatorum, J. Pediat. **19**:205 (Aug.) 1941. Javert, C. T., and Mann, C.: Prothrombin Concentration in Normal Pregnancy, Am. J. Obst. & Gynec. **42**:115 (Sept.) 1941. Maumenee, A. E.; Hellman, L. M., and Shettles, L. B.: Factors Influencing Plasma Prothrombin in New-Born Infants: Effect of Antenatal Administration of Vitamin K on Incidence of Retinal Hemorrhage in New Born, Bull. Johns Hopkins Hosp. **68**:158 (Feb.) 1941. Norris, R. F., and Bennett, M. C.: Plasma Prothrombin Values of Mothers and Infants at Delivery: Further Studies Including Comparative Values of Umbilical Arteries and Veins, Surg., Gynec. & Obst. **72**:758 (April) 1941. Stevens, R. J.: Vitamin K and Its Use in Prevention and Treatment of Hemorrhage, West Virginia M. J. **37**:206 (May) 1941.

41. de Beer, E. J.; Drektar, L., and Flusser, B.: Routes of Administration of Material Capable of Acting as Vitamin K, Proc. Soc. Exper. Biol. & Med. **46**:535 (April) 1941. Russell, H. K., and Page, R. C.: Effect of Topical Application of 2-Methyl-1,4-Naphthoquinone (Synthetic Vitamin K Analogue) on Prothrombin Level of New Born Infants, with Reference to Simplified Microprothrombin Test, Am. J. M. Sc. **202**:355 (Sept.) 1941.

42. Astrowe, P. S.; Palmerton, E. S., and Henderson, V.: Clinical Studies with Vitamin K in New-Born Infants, J. Pediat. **18**:507 (April) 1941. Huber, C. P., and Shrader, J. S.: Blood Prothrombin Levels in New Born, Am. J. Obst. & Gynec. **41**:566 (April) 1941. Mull, J. W.; Bill, A. H., and Skowronska, H.: Effect on New Born of Vitamin K Administered to Mothers in Labor, J. Lab. & Clin. Med. **26**:1305 (May) 1941. Valentine, E. H.; Reinhold, J. G., and Schneider, E.: Effectiveness of Prenatal Administration of 2-Methyl-1,4-Naphthoquinone in Maintaining Normal Prothrombin Levels in Infants, Am. J. M. Sc. **202**:359 (Sept.) 1941.

been obtained in infants after intravenous injection in mothers only ten minutes before delivery.[43] The administration of vitamin K to women at term, therefore, is rapidly becoming a routine procedure for the protection of infants and for the prevention of neonatal hemorrhage.

Reports of clinical experience continue to be uniformly enthusiastic regarding the value of vitamin K in the treatment of cholemic bleeding and the other manifestations of a deficiency of prothrombin associated with jaundice and hepatic disease.[44]

Observers are in agreement that in a small proportion of cases there is no response to treatment with vitamin K, even though the latter is administered intravenously or intramuscularly. In such cases evidence of marked hepatic damage has been found.[45] The failure of a patient

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43. Bohlender, G. P.; Rosenbaum, W. M., and Sage, E. C.: Antepartum Use of Vitamin K in the Prevention of Prothrombin Deficiency in the New Born, J. A. M. A. **16**:1763 (April 19) 1941.

44. Quick, A. J.: Therapeutic Value and Limitation of Vitamin K, Nebraska M. J. **26**:1 (Jan.) 1941. Anderson, E. R.; Karabin, J. E.; Udesky, H. L., and Seed, L.: Oral Administration of Synthetic Vitamin K (2-Methyl-1,4-Naphthoquinone), Surgery **9**:361 (March) 1941. Zenker, R., and Meurer, H.: Vitamin K in Surgery, München. med. Wchnschr. **88**:622 (May 30) 1941. Coller, F. A., and Farris, J. M.: Management of Jaundiced Patient with Special Reference to Vitamin K, Surg., Gynec. & Obst. **73**:21 (July) 1941. Tourinho, R.: Vitamin K in Surgery, Hospital, Rio de Janeiro **19**:755 (May) 1941.

45. Warner, E. D.: Plasma Prothrombin: Effect of Partial Hepatectomy, J. Exper. Med. **68**:831, 1938. Bollman, J. L.; Butt, H. R., and Snell, A. M.: The Influence of the Liver on the Utilization of Vitamin K, J. A. M. A. **115**:1087 (Sept. 28) 1940. Brinkhous, K. M., and Warner, E. D.: Effect of Vitamin K on Hypoprothrombinemia of Experimental Liver Injury, Proc. Soc. Exper. Biol. & Med. **44**:609 (June) 1940. Andrus, W. D.; Lord, J. W., Jr., and Moore, R. A.: The Effect of Hepatectomy on the Plasma, Prothrombin and the Utilization of Vitamin K, Surgery **6**:899 (Dec.) 1939. Lord, J. W., Jr.; Andrus, W. D., and Moore, R. A.: Metabolism of Vitamin K and Role of the Liver in Production of Prothrombin in Animals, Arch. Surg. **41**:585 (Sept.) 1940. Rhoads, J. E.: Physiological Factors Regulating the Level of the Plasma Prothrombin, Ann. Surg. **111**:916 (May) 1940. Allen, J. G., and Julian, O. C.: Clinical Use of a Synthetic Substance Resembling Vitamin K (2-Methyl-1,4-Naphthoquinone), Arch. Surg. **40**:912 (May) 1940. Butt, H. R.; Snell, A. M.; Osterberg, A. E., and Bollman, J. L.: Treatment of Hypoprothrombinemia: Use of Various Synthetic Compounds Exhibiting Antihemorrhagic Activity (Vitamin K_1 Activity), Proc. Staff Meet., Mayo Clin. **15**:69 (Jan. 31) 1940. Scanlon, G. H.; Brinkhous, K. M.; Warner, E. D.; Smith, H., and Flynn, J. E.: Plasma Prothrombin and the Bleeding Tendency with Special Reference to Jaundiced Patients and Vitamin K Therapy, J. A. M. A. **112**:1898 (May 13) 1939. Butt, H. R., and Leary, W. V.: Diseases of Nutrition, Arch. Int. Med. **67**:411 (Feb.) 1941. Andrus, W. DeW.: The Newer Knowledge of Vitamin K, Bull. New York Acad. Med. **17**:116 (Feb.) 1941. Reid, J.: Prothrombin Deficiency in Disease of the Liver and Bile Passages and Its Treatment with Synthetic Vitamin K, Brit. M. J. **1**:579 (April) 1941. Lord, J. W., Jr., and Andrus, W. D.: Differentiation of Intrahepatic and Extra-

to respond to intensive therapy with vitamin K not only is of value in differential diagnosis but is of serious prognostic import. Because in some instances there seems to be confusion on this point, it should be emphasized that a prolonged prothrombin time in an untreated patient usually is an accompaniment of the jaundice and a consequence of the interference with the flow of bile into the intestine. It therefore is evidence of a deficiency of vitamin K and not a measure of hepatic injury. It is the failure of such a patient to respond to adequate and preferably parenteral therapy that indicates functional incapacity of the liver.

Soon after the isolation of the naturally occurring vitamin K_1 it was shown to have the chemical structure of 2-methyl-3-phytyl-1,4-naphthoquinone. This led to the testing of related chemical compounds, and menadione (2-methyl-1,4-naphthoquinone) was found to be one of the most active. Because of its activity and the ease with which it is prepared synthetically, it has largely replaced the natural vitamin K_1 in therapy. Menadione, while readily soluble in oil, has been considered too insoluble in water for intravenous or intramuscular injection. Water-soluble compounds possessing prothrombogenic properties have been sought, and several such compounds have been described and introduced into clinical medicine. Four-amino-2-methyl-1-naphthol hydrochloride, marketed by Parke, Davis & Company as synkamin;[46] 2-methyl-1,4-naphthohydroquinone diphosphoric acid ester tetra sodium salt, marketed by Hoffmann-Laroche Inc. as synkayvite,[47] and 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate, marketed by the Abbott Laboratories,[48] have been reported to be of clinical value.

It may be accepted that all these preparations are effective in the treatment of hypoprothrombinemia. When given by mouth they are

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hepatic Jaundice: Response of Plasma Prothrombin to Intramuscular Injection of Menadione (2-Methyl-1,4-Naphthoquinone) as a Diagnostic Aid, Arch. Int. Med. **68**:199 (Aug.) 1941. Stewart, J. D.: Clinical Significance of Prothrombin Deficiency and Its Treatment, Ann. Surg. **114**:907 (Nov.) 1941. Tocantins, L. M., and Jones, H. W.: Hypoprothrombinemia: Effect of Peroral and Parenteral Administration of Synthetic Vitamin K Substitute (2-Methyl-1,4-Naphthoquinone), ibid. **113**:276 (Feb.) 1941. Ziffren, S. E.; Owen, C. A.; Warner, E. D., and Peterson, T. R.: Hypoprothrombinemia and Liver Function, Surg., Gynec & Obst. **74**:463 (Feb., no. 2A) 1942.

46. Sharp, E. A.; von der Heide, E. G., and Good, W. H.: Vitamin K Activity of 2-Methyl-1,4-Naphthoquinone and 4-Amino-2-Methyl-1-Naphthol in Hypoprothrombinemia, J. Lab. & Clin. Med. **26**:818 (Feb.) 1941.

47. Lee, J.; Solmssen, U. V.; Steyermark, A., and Foster, R. H. K.: Antihemorrhagic Activity of Tetra Sodium 2-Methyl-1,4-Naphthohydroquinone Diphosphoric Acid Ester and Other Naphthoquinone Derivatives, Proc. Soc. Exper. Biol. & Med. **45**:407 (Oct.) 1940. Almquist, H. J., and Klose, A. A.: Comparative Activities of Certain Antihemorrhagic Compounds, ibid. **45**:55 (Oct.) 1940.

48. Davison, M.; Steigmann, F., and Udesky, H. L.: Clinical Studies on the Antihemorrhagic Effects of a New Water-Soluble Vitamin K-Like Substance, Surg., Gynec. & Obst. **74**:35 (Jan.) 1942.

readily absorbed without the simultaneous administration of bile salts. They may be given subcutaneously or intravenously when parenteral therapy is indicated. Olwin [49] reports that a satisfactory therapeutic response was obtainable with each of these preparations. He found in addition that menadione was sufficiently soluble in distilled water to permit the preparation of a 0.01 per cent solution. He gave this solution intravenously in doses of 0.1 to 12 mg., with satisfactory therapeutic responses. To date, there would seem to be little evidence to indicate a basis for choice between these different compounds.

EFFECT OF DIET ON THE LIVER

Not only does the character of the diet determine the work performed by the liver in the metabolism and oxidation of the food, but it may have an effect on the ability of the liver to carry out that work. Greene and Farrell,[28] Madden and Whipple,[50] Hawkins [51] and Mann [52] have summarized the recent literature on the role of the liver in protein metabolism and in the production and utilization of the plasma proteins. The importance of an adequate supply of protein in the diet to maintain reserves of protein in the liver and to increase its resistance to injury by various toxic agents has been emphasized.

Most investigators are in agreement that an undue increase in the amount of fat present in the liver contributes to the development of cirrhosis. The relation of the liver to fat metabolism has been reviewed recently by Greene, Handelsman and Babey,[1] Best and Ridout [53] and Channon.[54] Many different factors acting either singly or in combination may produce an increase in the amount of fat in the liver. Feeding a high fat diet will produce a permanent increase, which contrasts with the temporary increase produced by fasting. Overfeeding even of a high carbohydrate diet may produce a fatty liver in consequence of the production of fat from carbohydrate. This is particularly true in birds, such as geese.[55] Thiamine, or vitamin B_1, will increase the

49. Olwin, J. H.: The Intravenous Use of Vitamin K, J. A. M. A. **117**:432 (Aug. 9) 1941.

50. Madden, S. C., and Whipple, G. H.: Plasma Proteins: Their Source, Production and Utilization, Physiol. Rev. **20**:194 (April) 1940.

51. Hawkins, W. B.: Liver and Bile, in Luck, J. M., and Hall, V. E.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1941, vol. 3, p. 259.

52. Mann, F. C.: The Liver and Medical Progress, J. A. M. A. **117**:1577 (Nov. 8) 1941.

53. Best, C. H., and Ridout, J. H.: Choline as a Dietary Factor, in Luck, J. M., and Smith, J. H. C.: Annual Review of Biochemistry, Stanford University, Calif., Annual Reviews, Inc., 1939, vol. 8, p. 439.

54. Channon, J. H.: Fat Metabolism, in Luck, J. M., and Smith, J. H. C.: Annual Review of Biochemistry, Stanford University, Calif., 1940, vol. 9, p. 231.

55. Flock, E. V.; Bollman, J. L.; Hester, H. R., and Mann, F. C.: Fatty Liver in the Goose Produced by Overfeeding, J. Biol. Chem. **121**:117 (Oct.) 1937.

amount of fat in the liver under certain experimental conditions by stimulating the formation of fat from carbohydrate.[56] Pyridoxine, or vitamin B_6, and biotin, or vitamin H, have been implicated in increasing the fat in the liver. The chronic fatty infiltration of the liver reported in pancreatectomized dogs and some patients with diabetes is well recognized. The use of extracts of anterior pituitary will produce a fatty liver.[57] Diethylstilbestrol and some other estrogens have been reported to produce fatty livers, though this is denied by other investigators.[58] The concentration of cholesterol in the liver tends to vary with the neutral fat, though it is deposited to a lesser degree, but if cholesterol is fed then it may become the predominant lipid in the liver.[59]

An increase in the amount of protein or carbohydrate in the diet usually decreases the amount of fat in the liver. Choline [60] or pancreatic extract [61] will prevent the accumulation of fat due to a high fat diet. Lipocaic has been described as the fat-metabolizing hormone of the pancreas, but its physiologic status is still undecided.[62]

56. McHenry, E. W., and Gavin, G.: The Effect of Several B Vitamins and of Choline upon Liver and Body Fat, J. Biol. Chem. **128**:66 (June) 1939; The B Vitamins and Fat Metabolism: IV. The Synthesis of Fat from Protein, ibid. **138**:471 (April) 1941; The Effect of Biotin upon the Synthesis of Lipids in Rats, ibid. **140**:lxxxvii (July) 1941.

57. Mackay, E. M., and Barnes, R. H.: Choline and Pancreas Extract on Fatty Livers and Ketosis Due to Anterior Pituitary Extract, Proc. Soc. Exper. Biol. & Med. **38**:803 (June) 1938.

58. Selye, H.: On the Toxicity of Estrogens with Special Reference to Diethylstilbestrol, Canad. M. A. J. **41**:48 (July) 1939. Shorr, E.; Robinson, F. H., and Papanicolaou, G. N.: A Clinical Study of the Synthetic Estrogen Stilbestrol, J. A. M. A. **113**:2312 (Dec. 23) 1939. Gumbrecht, P., and Loeser, A.: Ueber das Auftreten von Leberschädigungen nach peroraler Zufuhr kunstlicher Brunstoffe, Klin. Wchnschr. **18**:1195 (Sept. 2) 1939. MacBryde, C. M.; Castrodale, D.; Loeffel, E., and Freedman, H.: The Synthetic Estrogen Diethylstilbestrol: Clinical and Experimental Studies, J. A. M. A. **117**:1240 (Oct. 11) 1941. Teague, R. S.: The Effect of Estrogens on the Microscopic Appearance of the Liver, ibid. **117**:1242 (Oct. 11) 1941. Aaron, A. H.; Meyers, F.; Lipsitz, M. H., and Hubbard, R. S.: Toxicity Studies on Stilbestrol, Am. J. Digest. Dis. **8**:437 (Nov.) 1941.

59. Cook, R. P., and McCullagh, G. P.: A Comparative Study of Cholestrol Metabolism and Its Relation to Fatty Infiltration, with Particular Reference to Experimental Cholestrol Atheroma, Quart. J. Exper. Physiol. **29**:283 (Aug.) 1939.

60. Best, C. H.; Ferguson, G. C., and Hershey, J. M.: Choline and Liver Fat in Diabetic Dogs, J. Physiol. **79**:94 (July 28) 1933.

61. Montgomery, M. L.; Entenman, C.; Chaikoff, I. L., and Nelson, C.: The Role of the External Secretion of the Pancreas in Lipid Metabolism: The Prevention of Fatty Livers in Depancreatized and Duct-Ligated Dogs by the Daily Feeding of Fresh Pancreatic Juice, J. Biol. Chem. **137**:693 (Feb.) 1941.

62. Dragstedt, L. R.; Van Prohaska, J., and Harms, H. P.: Observations on a Substance in Pancreas (a Fat Metabolizing Hormone) Which Permits Survival

(Footnote continued on next page)

That prolonged fatty infiltration of the liver is harmful and may be one of the factors responsible for the development of cirrhosis is accepted. Much of the literature was reviewed by Connor [63] in his study of the relation of fatty livers to alcoholism and their role in the development of alcoholic cirrhosis. This topic was reviewed by Greene and Hotz.[37a]

Chloroform and carbon tetrachloride have been the favored poisons for the experimental production of injury to the liver. Other poisons, however, are being used. McCulloch [64] reports that the admixture of seeds of the yellow tarweed Amsinckia intermedia with the feed will produce cirrhosis of the liver in domestic animals. It has not yet been tried in the smaller laboratory animals.

Foodstuffs grown on soils rich in selenium have been found to be toxic. This has been shown to be due to the passage of selenium from the soil into forage or grains raised on it. The symptoms and pathologic changes found in experimental animals with chronic selenium poisoning are various, but atrophic nodular cirrhosis of the liver is reported in cases of more severe poisoning.[65] The experimental studies of Smith and associates [66] and Lewis, Schultz and Gortner [67] have shown that

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and Prevents Liver Changes in Depancreatized Dogs, Am. J. Physiol. **117**:175-181 (Sept.) 1936. Dragstedt, L. R.: Present Status of Lipocaic, J. A. M. A. **114**:29 (Jan. 6) 1940. Vermeulen, C.; Dragstedt, L. R.; Clark, D. E.; Julian, O. C., and Allen, J. G.: Effect of the Administration of Lipocaic and Cholesterol in Rabbits, Arch. Surg. **44**:260-267 (Feb.) 1942. Gavin, G., and McHenry, E. W.: B. Vitamins and Fat Metabolism Synthesis of Fat from Protein, J. Biol. Chem. **141**:619 (Nov.) 1941. Isabolinskaya, R. M.: The Lipocaic of the Pancreas and the Fat-Carbohydrate Metabolism, Bull. biol. et méd. expér. URSS **9**:107, 1940.

63. Connor, C. L.: Fatty Infiltration of the Liver and the Development of Cirrhosis in Diabetes and Chronic Alcoholism, Am. J. Path. **14**:347 (May) 1938. Connor, C. L.: The Etiology and Pathogenesis of Alcoholic Cirrhosis of the Liver, J. A. M. A. **112**:387 (Feb. 4) 1939.

64. McCulloch, E. C.: The Experimental Production of Hepatic Cirrhosis by the Seed of Amsinckia Intermedia, Science **91**:95 (Jan. 26) 1940.

65. Smith, M. I., and Westfall, B. B.: Further Field Studies on the Selenium Problem in Relation to Public Health, Pub. Health Rep. **52**:1375 (Oct. 1) 1937.

66. Smith, M. I.: The Influence of Diet on the Chronic Toxicity of Selenium, Pub. Health Rep. **54**:1441 (Aug. 4) 1939. Lillie, R. D., and Smith, M. I.: Histogenesis of Hepatic Cirrhosis in Chronic Food Selenosis, Am. J. Path. **16**:223 (March) 1940. Smith, M. I., and Stohlman, E. F.: Further Observations on Influence of Dietary Protein on Toxicity of Selenium, J. Pharmacol. & Exper. Therap. **70**:270 (Nov.) 1940. Smith, M. I.: Chronic Endemic Selenium Poisoning: A Review of the More Recent Field and Experimental Studies, J. A. M. A. **116**: 562 (Feb. 15) 1941.

67. Lewis, H. B.; Schultz, J., and Gortner, R. A., Jr.: Dietary Protein and Toxicity of Sodium Selenite in White Rats, J. Pharmacol. & Exper. Therap. **68**:292 (Feb.) 1940. Gortner, R. A., Jr.: Chronic Selenium Poisoning of Rats as Influenced by Dietary Protein, J. Nutrition **19**:105 (Feb.) 1940.

selenium was most toxic when the experimental animals were fed a low protein, high carbohydrate diet. The toxic symptoms were much less marked when a high protein diet was fed. A high fat diet was not as harmful as was the low protein diet.

Arsenic has an injurious effect on the liver. Messinger and Hawkins [68] report that a high protein diet would protect dogs against toxic doses of arsphenamine. Carbohydrate was less beneficial, while a high fat diet was harmful. Von Glahn, Flinn and Keim [69] fed sodium arsenate to rabbits on a diet of hay and oats and were able to produce hepatic necrosis and mild degrees of cirrhosis. Carbohydrates in the form of white bread and potatoes reduced the degree of hepatic injury. Brewers' yeast also reduced the severity of the damage and prolonged the life of the experimental animals.

Evidence is being accumulated that dietary factors other than the relative amounts of carbohydrate, protein and fat in the diet affect the liver. György and Goldblatt [70] observed that when young rats were kept on a diet devoid of vitamin B but supplemented with thiamine, riboflavin and pyridoxine they showed evidence of hepatic injury. This consisted primarily of acute diffuse necrosis with fatty infiltration. In some instances considerable periportal fibrosis was observed. Rats fed on the same basal diet supplemented with yeast or yeast extract did not show any pathologic changes in the liver. Shortly afterward, Rich and Hamilton [71] reported the development of cirrhosis of the liver on a nutritional basis in rats, while Spellberg, Keeton and Ginsberg [72] made similar observations in guinea pigs. In both studies the addition of

68. Messinger, W. J., and Hawkins, W. B.: Arsphenamine Liver Injury Modified by Diet Protein and Carbohydrate Protective but Fat Injurious, Am. J. M. Sc. **199**:216 (Feb.) 1940.

69. von Glahn, W. C.; Flinn, F. B., and Keim, W. F., Jr.: Effect of Certain Arsenates on the Liver, Arch. Path. **25**:488 (April) 1938. von Glahn, W. C., and Flinn, F. B.: The Effect of Yeast on the Incidence of Cirrhosis Produced by Lead Arsenate, Am. J. Path. **15**:771 (Nov.) 1939.

70. György, P., and Goldblatt, H.: Hepatic Injury on a Nutritional Basis in Rats, J. Exper. Med. **70**:185 (Aug.) 1939; Experimental Production of Dietary Liver Injury (Necrosis, Cirrhosis) in Rats, Proc. Soc. Exper. Biol. & Med. **46**:492 (March) 1941.

71. Rich, A. R., and Hamilton, J. D.: The Experimental Production of Cirrhosis of the Liver by Means of a Deficient Diet, Bull. Johns Hopkins Hosp. **66**:185 (March) 1940.

72. Spellberg, M. A., and Keeton, R. W.: The Production of Fatty and Fibrotic Livers in Guinea Pigs and Rabbits by Seemingly Adequate Diets, Am. J. M. Sc. **200**:688 (Nov.) 1940. Spellberg, M. A.; Keeton, R. W., and Ginsberg, R.: Dietary Production of Hepatic Cirrhosis in Rabbits with an Analysis of the Factors Involved, Arch. Path. **33**:204 (Feb.) 1942.

yeast to the diet prevented the development of the changes in the liver. The earlier studies of Curtis and Newburgh and others [73] had shown that an excess of cystine in the diet would produce acute hepatic necrosis. Studies by György, Poling and Goldblatt [74] and Blumberg and McCollum [75] showed that the addition of extra cystine to the experimental diet of rats accentuated the development of cirrhosis. Yeast or choline prevented the cirrhosis even though extra cystine was fed. Similar results are reported by Lillie, Daft and Sebrell,[76] who produced cirrhosis in rats by the use of a diet low in protein and fat with added cystine. The cirrhosis was prevented by feeding choline, methionine or casein, either singly or in combination. Supplying 20 per cent alcohol to the rats in place of drinking water apparently had no influence on the results.

The clinical counterpart of some of these experimental studies is presented by Patek and Post.[77] They emphasize the frequency in cases of portal cirrhosis of alcoholism, a deficient diet, loss of weight, malnutrition and a specific deficiency of the vitamin B complex. This latter was shown by the frequency of polyneuritis, "pellagrous" dermatitis and atrophy of the lingual papillae. A group of 54 patients with portal cirrhosis therefore were placed on a highly nutritious diet together with vitamin B complex. This diet contained 149 Gm. of protein, 175 Gm. of fat and 365 Gm. of carbohydrate. This was supplemented by the

73. Curtis, A. C., and Newburgh, L. H.: The Toxic Action of Cystine on the Liver of the Albino Rat, Arch. Int. Med. **39**:828 (June) 1927. Sullivan, M. X.; Hess, W. C., and Sebrell, W. H.: Studies on Biochemistry of Sulphur: Preliminary Studies on Amino-Acid Toxicity and Amino-Acid Balance, Pub. Health Rep. **47**:75 (Jan. 8) 1932. Lillie, R. D.: Histopathological Changes Produced in Rats by Addition to Diet of Various Amino Acids (Glycine, Lysine, Tryptophane, Cystine, Tyrosine and Glutamic Acid) and of Mixtures of Some of Them, ibid. **47**:83 (Jan. 8) 1932. Earle, D. P., Jr., and Victor, J.: Cirrhosis of the Liver Caused by Excess Dietary Cystine, J. Exper. Med. **73**:161 (Feb.) 1941.

74. György, P.; Poling, C. E., and Goldblatt, H.: Necrosis, Cirrhosis and Cancer of Liver in Rats Fed a Diet Containing Dimethylaminoazobenzene, Proc. Soc. Exper. Biol. & Med. **47**:41 (June) 1941.

75. Blumberg, H., and McCollum, E. V.: The Prevention by Choline of Liver Cirrhosis in Rats on High Fat, Low Protein Diets, Science **93**:598 (June 29) 1941.

76. Lillie, R. D.; Daft, F. S., and Sebrell, W. H., Jr.: Cirrhosis of the Liver in Rats on a Deficient Diet and the Effect of Alcohol, Pub. Health Rep. **56**:1255 (June 13) 1941. Daft, F. S.; Sebrell, W. H., and Lillie, R. D.: Production and Apparent Prevention of a Dietary Liver Cirrhosis in Rats, Proc. Soc. Exper. Biol. & Med. **48**:228 (Oct.) 1941.

77. Patek, A. J., Jr.: Treatment of Alcoholic Cirrhosis of the Liver with High Vitamin Therapy, Proc. Soc. Exper. Biol. & Med. **37**:329 (Nov.) 1937. Patek, A. J., Jr., and Post, J.: Treatment of Cirrhosis of the Liver by a Nutritious Diet and Supplements Rich in Vitamin B Complex, J. Clin. Investigation **20**:481 (Sept.) 1941.

addition of 50 Gm. of brewers' yeast and 5 mg. of thiamine hydrochloride daily. Twice weekly 5 cc. of concentrated liver extract (15 U. S. P. units per cubic centimeter) was given intramuscularly. The control series was obtained by a study of the records of 386 patients with cirrhosis treated in various metropolitan hospitals. The initial course of the disease before the institution of treatment was similar in the two groups of patients.

The response of the patients treated by Patek and Post was much more satisfactory than was that of the control series. After the onset of ascites 72 per cent of the treated patients were alive for more than six months, while only 57 per cent of the control patients survived for this period. At the end of the second year, 45 per cent of the treated patients were alive as compared to 21 per cent of the control series. In addition to an increased period of survival, there were signs of general physical improvement. In a significant number of cases the disappearance of ascites, edema, jaundice or vascular spiders was noted. The level of the serum albumin and globulin returned toward normal, while the Takata-Ara, the cephalin flocculation and the bromsulphalein tests showed improvement.[78]

Closely related to the studies of the production of cirrhotic changes in the liver by dietary means are the studies of the effects of diet on the production of experimental cancer of the liver. The demonstration that cancer of the skin could be produced by the persistent application of gas works tar [79] led to a hunt for carcinogenic substances. It is now known that many chemical compounds have carcinogenic properties.[80] One of these is the dye N-N dimethylaminoazobenzene (butter yellow). If the dye is fed to rats on a diet of unpolished rice and carrots, a primary cancer of the liver is produced.[81] Adding dried liver, yeast or riboflavin and casein to this regimen prevents the development of the cancer. Methionine, cystine and choline likewise have a protective

78. Patek, A. J., Jr.; Post, J., and Victor, J. C.: The Vascular "Spider" Associated with Cirrhosis of the Liver, Am. J. M. Sc. **200**:341 (Sept.) 1940. Hanger, F. M., and Patek, A. J., Jr.: The Cephalin Flocculation Test in Cirrhosis of the Liver, ibid. **202**:48 (July) 1941. Post, J., and Patek, A. J., Jr.: Serum Proteins in Cirrhosis of the Liver: I. Relation to Prognosis and to Formation of Ascites, Arch. Int. Med. **69**:67 (Jan.) 1942; II. Nitrogen Balance Studies on Five Patients, ibid. **69**:83 (Jan.) 1942.

79. Yamagiwa, K., and Ichikawa, K.: Experimentelle Studien über die Pathogenese der Epithelialgeschwulste, Mitt. a. d. med. Fak. d. k. Univ. zu Tokyo **15**:295, 1916.

80. Kinosita, R.: The Changes in the Liver of the Rat Caused by 4-Dimethylaminobenzene-1-Azo-1-Naphthalene, and Related Compounds, Gann **34**:164-167 (June) 1940.

81. Kinosita, R.: Studies on the Cancerogenic Azo and Related Compounds, Yale J. Biol. & Med. **12**:287 (Jan.) 1940.

effect.[82] On the other hand, the recent experiments of du Vigneaud and his associates [83] and of György, Landy and Goldblatt [84] are of especial interest in showing that biotin (vitamin H) increases the carcinogenic action of the butter yellow.

The first changes in the liver of a rat on this carcinogenic regimen are necrosis and fibrosis suggestive of cirrhosis. The characteristic hepatoma develops later. The similarity between many of the diets used in the experimental production of carcinoma and those used in the production of experimental cirrhosis is of great interest. The clinical experience that hepatoma or primary cancer of the liver develops most commonly on the basis of a preexisting cirrhosis is intriguing.

Rhoads [85] has attempted to study the chemical processes in the cells of the normal liver and the changes in the presence of cancer. Particular attention was paid to the various kinetic enzyme systems with which the different vitamins are associated. These studies indicated that when the rats were on a diet deficient in riboflavin, the administration of butter yellow stimulated the excretion of reserve riboflavin in the urine and so increased the severity of the deficiency. Studies of coenzyme I, a diphosphopyridine nucleotide, which contains nicotinic acid as an essential constituent, showed that the livers of rats on a carcinogenic diet contained normal amounts of coenzyme I. If butter yellow was added to the diet, there was a prompt and serious decrease in the amount of coenzyme I. Tumor tissue contained even smaller amounts of

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82. Ando, T.: Experimentelle Leberkarzinomentstehung und Getreide, Gann **32**:252 (June) 1938. Nakahara, W.; Mori, K., and Fujiwara, T.: Inhibiting Effect of Yeast Feeding on Experimental Production of Liver Cancer (Preliminary Note), ibid. **32**:465 (Dec.) 1938; Does Vitamin B_1 Inhibit Experimental Production of Liver Cancer? Second Preliminary Note on Effect of Diet on Experimental Production of Liver Cancer, ibid. **33**:13 (Feb.) 1939; Inhibiting Effect of Yeast Feeding on Experimental Production of Liver Cancer, ibid. **33**:57 (April) 1939; Inhibition of Experimental Production of Liver Cancer by Liver Feeding—Study in Nutrition, ibid. **33**:406 (Oct.) 1939. Morigami, S., and Kasiwabara, N.: Inhibition of the Experimental Production of Liver Cancer by Millet Feeding, ibid. **35**:65 (April) 1941. Sugiura, K., and Rhoads, C. P.: Experimental Liver Cancer in Rats and Its Inhibition by Rice-Bran Extract, Yeast and Yeast Extract, Cancer Research **1**:3 (Jan.) 1941. Kensler, C. J.; Sugiura, K.; Young, N. F.; Halter, C. R., and Rhoads, C. P.: Partial Protection of Rats by Riboflavin with Casein Against Liver Cancer Caused by Dimethylaminoazobenzene, Science **93**:308 (March 28) 1941.

83. du Vigneaud, V.; Spangler, J. M.; Burk, D.; Kensler, C. J.; Sugiura, K., and Rhoads, C. P.: The Procarcinogenic Effect of Biotin in Butter Yellow Tumor Formation, Science **95**:174 (Feb. 13) 1942.

84. György, P.; Landy, and Goldblatt, H., cited by du Vigneaud and others.[83]

85. Rhoads, C. P.: Recent Studies in the Production of Cancer by Chemical Compounds: The Conditioned Deficiency as a Mechanism, Bull. New York Acad. Med. **18**:53 (Jan.) 1942.

coenzyme I than the damaged hepatic cells. Rhoads therefore argues that these observations constitute evidence that the administration of one carcinogenic chemical injures normal hepatic cells by interfering with enzyme systems which are essential for normal chemical function and so for normal life of the cells. Further discussion at the present time is speculative, but these studies promise to open the way for a better understanding of cell function, especially in the liver.

THE LIVER AND VITAMIN A

Reference was made a year ago to the accumulating evidence which indicates that there is some deficiency of vitamin A in patients with hepatic disease. Most of these reports, such as the recent studies of Wohl and Feldman and others,[86] are limited to the demonstration of an abnormal visual adaptation to darkness. Such a method, while accepted as satisfactory for the clinical recognition of deficiency of vitamin A, is not applicable to experimental animals.

The introduction of chemical methods of assay for vitamin A has permitted the study of the storage of the vitamin in various organs. The earlier studies of Baumann, Riising and Steenbock and others [87] indicated that the predominant proportion of any surplus intake over the basal requirement is stored in the liver. These studies have been confirmed by Kao and Sherman.[88] Drummond and his associates [89] found the administered vitamin A was uniformly distributed through

86. Wohl, M. G., and Feldman, J. B.: The Occurrence of Avitaminosis A in Diseases of the Liver, Am. J. Digest. Dis. **8**:464 (Dec.) 1941. Jeans, P. C.; Blanchard, E. L., and Satterthwaite, F. E.: Dark Adaptation and Vitamin A: Further Studies with the Biophotometer, J. Pediat. **18**:170 (Feb.) 1941. Eckhardt, R. E., and Johnson, L. V.: A Comparison of Two Methods of Measuring Dark Adaptations, ibid. **18**:195 (Feb.) 1941. Bajardi, G., and Galeone, A.: La cecità notturna quale sintomo di malattia epatica, Policlinico (sez. prat.) **48**:193 (Feb. 3) 1941.

87. Baumann, C. A.; Riising, B. M., and Steenbock, H.: Fat-Soluble Vitamins: The Absorption and Storage of Vitamin A in the Rat, J. Biol. Chem. **107**:705 (Dec.) 1934. Moore, T.: Vitamin A and Carotene: The Distribution of Vitamin A and Carotene in the Body of the Rat, Biochem. J. **25**:275, 1931. McCoord, A. B., and Luce-Clausen, E. M.: Storage of Vitamin A in Liver of Rat, J. Nutrition **7**:557 (May) 1934. Chevallier, A., and Choron, Y.: Sur la teneur du foie en vitamine A et ses variations, Compt. rend. Soc. de biol. **120**:1223, 1935.

88. Kao, H. C., and Sherman, H. C.: Influence of Nutritional Intake upon Concentration of Vitamin A in Body Tissues, Proc. Soc. Exper. Biol. & Med. **45**:589 (Nov.) 1940.

89. Drummond, J. C.; Gilding, H. P., and Macwalter, R. J.: Fate of Carotene Introduced into Circulation, J. Physiol. **82**:75 (Aug. 24) 1934. Drummond, J. C., and Macwalter, R. J.: Fate of Carotene Injected into Circulation of Rat, ibid. **83**:236 (Dec. 31) 1935.

the liver. Schneider and Widmann [90] found that the thyrotropic hormone caused a discharge of vitamin A from the liver. Young and Wald [91] found further that the removal of one lobe of the liver brought about a loss of the vitamin from the remaining lobes. This loss was accompanied by an increase of the vitamin in the blood. Stimulation of the splanchnic nerves increased the level of the vitamin in the blood, but stimulation of the middle cervical sympathetic ganglion was without effect. Young and Waldo concluded, therefore, that not only is the liver a storehouse of vitamin A but the vitamin may be mobilized in a manner similar to that in which sugar is mobilized by the liver.

The correlation between the concentration of vitamin A in the blood and the stores in the liver was emphasized by Lewis and his associates [92] and by Horton, Murrill and Curtis [93] on the basis of feeding experiments in rats.

Confirmatory clinical data are provided by several investigators,[94] including Cox and Ralli and her associates, who found much less vitamin A in the livers of patients with cirrhosis than they did in the livers of normal subjects. Not only was the concentration of vitamin A in the plasma reduced in the presence of cirrhosis, but when vitamin A was administered orally in the form of cod liver oil concentrates, the resultant rise in the blood level was much less in patients with cirrhosis than it was in normal subjects.[95]

That a reduction in the concentration of vitamin A in the plasma is not necessarily indicative of cirrhosis or hepatic injury was found

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90. Schneider, E., and Widmann, E.: Ueber Beziehungen des Vitamin A und seiner Vorstufen zur Leberschädigung und zur Widerstandskraft gegenüber Infektionen: VII. Mitteilung der fortgesetzten Untersuchungen über die Hyperthyreosen, Klin. Wchnschr. **13**:1497 (Oct. 20) 1934.

91. Young, G., and Wald, G.: Mobilization of Vitamin A by Sympathico-Adrenal System, Am. J. Physiol. **131**:210 (Nov.) 1940.

92. Lewis, J. M.; Bodansky, O.; Falk, K. G., and McGuire, G.: Relationship on Vitamin A Blood Level in the Rat to Vitamin A Intake and to Liver Storage, Proc. Soc. Exper. Biol. & Med. **46**:248 (Feb.) 1941.

93. Horton, P. B.; Murrill, W. A., and Curtis, A. C.: Vitamin A and Carotene: I. The Determination of Vitamin A in the Blood and Liver as an Index of Vitamin A Nutrition of the Rat, J. Clin. Investigation **20**:387 (July) 1941.

94. Breusch, F., and Scalabrino, R.: Die quantitiven Verhältnisse der Leberlipoide, Ztschr. f. d. ges. exper. Med. **94**:569, 1934. Moore, T.: The Vitamin Reserve of the Adult Human Being in Health and Disease, Biochem. J. **31**:155, 1937. Cox, A. J.: Vitamin A Storage in Active and Arrested Cirrhosis, Am. J. Path. **15**:647 (Sept.) 1939. Ralli, E. P.; Papper, E.; Paley, K., and Bauman, E.: The Vitamin A and Carotene Content of Normal and Pathological Human Livers, Arch. Int. Med. **68**:102 (July) 1941.

95. Lasch, F.: Ueber den Vitamin A-Spiegel im Blute bei Leberkrankheiten, Klin. Wchnschr. **17**:1107 (Aug. 6) 1938. Ralli, E. P.; Bauman, E., and Roberts, L. B.: The Plasma Levels of Vitamin A After the Ingestion of Standard Doses: Studies in Normal Subjects and Patients with Cirrhosis of the Liver, J. Clin. Investigation **20**:709 (Nov.) 1941.

by Rhoads and his associates.[96] They report that 85 per cent of patients with cancer of the gastrointestinal tract had abnormally low levels of vitamin A in the plasma. Normal concentrations were found in the liver. Cox [97] reports 3 cases of massive necrosis of the liver in which it was possible to compare the concentration of vitamin A in normal and in damaged areas of the same liver. The quantity of vitamin was greater in areas of healing necrosis in which the hepatic cells had been destroyed. Cox, therefore, is in agreement with Lasch and Roller [98] in assuming that the greater part of the storage of vitamin A in the liver is in the Kupffer cells. In the cases reported by Cox, the Kupffer cells had been spared by the agent responsible for the necrosis of the parenchymal cells of the liver.

When frozen sections of tissues are examined with ultraviolet rays, they show a striking green fluorescence, which gradually fades. Popper and Greenberg [99] have demonstrated that this characteristic fluorescence is due to vitamin A. They have been able by this means to visualize the course of vitamin A metabolism in the tissues. The characteristic fluorescence was found in the epithelial and Kupffer cells of the liver, the fasicular layer of the adrenal, the interstitium of the kidney and the lung, the interstitial cell cords and the corpora lutea of the ovary, the pleura, the pericardium, the peritoneum, the meninges, the retina and the pigmented layer of the eye, fat cells and, after ingestion of vitamin A, in the upper part of the small intestine. The amount of fluorescent material in the tissues was reduced by removing vitamin A from the diet and increased by feeding an excess of the vitamin.

The livers of animals deficient in the vitamin showed none of the characteristic fluorescence. In the livers of normal animals the parenchymal cells showed fluorescence in small lipid droplets along the edge of the cytoplasm marking the boundary between the liver cells and the sinusoids. The cytoplasm also had a dimmer fluorescence, indicating a diffuse distribution of minute amounts of vitamin A. The Kupffer cells contained small lipid droplets rich in vitamin A. After ingestion

96. Abels, J. C.; Gerham, A. T.; Pack, G. T., and Rhoads, C. P.: Metabolic Studies in Patients with Gastro-Intestinal Cancer: III. The Hepatic Concentrations of Vitamin A, Proc. Soc. Exper. Biol. & Med. **48**:488 (Nov.) 1941.

97. Cox, A. J.: Site of Vitamin A Storage in the Liver, Proc. Soc. Exper. Biol. & Med. **47**:333 (June) 1941.

98. Lasch, F.: Vitamin A-Stoffwechsel und Leber bei Experimenteller Phosphorvergiftung, Klin. Wchnschr. **14**:1070 (July 27) 1935. Lasch, F., and Roller, D.: Ueber die Beeinflussung des Vitamin A-Stoffwechsels der Leber durch Blockade des Reticuloendothels, ibid. **15**:1636 (Nov. 7) 1936.

99. Popper, H.: Histological Demonstration of Vitamin A in Rats by Means of Fluorescence Microscopy, Proc. Soc. Exper. Biol. & Med. **43**:133 (Jan.) 1940; Vitamin A: The Distribution of Vitamin A in the Body, J. Mt. Sinai Hosp. **7**:119 (Sept.-Oct.) 1940. Popper, H., and Greenberg, R.: Visualization of Vitamin A in Rat Organs by Fluorescence Microscopy, Arch. Path. **32**:11 (July) 1941.

of vitamin A, there was not much change in the hepatic cells, but the Kupffer cells were loaded with the vitamin and produced enough fluorescence for it to be visible to the unaided eye.

Popper and associates [100] extended these studies to observations on a series of specimens of human liver obtained either at autopsy or by biopsy. The content of vitamin A in the liver was reduced in acute hepatitis and in cirrhosis. A less marked degree of reduction was observed in obstructive jaundice. The degree of fluorescence of the Kupffer cells and of the parenchymal cells generally paralleled each other.

In localized areas of hepatic necrosis the necrotic area was free from vitamin A, while the Kupffer cells in the surrounding areas were rich in the vitamin. In toxic edema associated with sepsis, in uremia and in diabetes the Kupffer cells were strongly fluorescent, while the liver cells showed only traces of the vitamin. If the damage to the liver was severe, the Kupffer cells were free from the vitamin.

Tumors of the liver were found by Popper and Ragins [101] to show a moderate degree of fluorescence. The Kupffer cells in these same tumors all showed the presence of vitamin A. Hepatic metastases which were free of vitamin A at their primary site did not show the characteristic fluorescence, though the surrounding liver tissue was rich in vitamin A.

On the basis of these studies Popper suggests that the amount of vitamin A in the liver was determined primarily by the functional state of the hepatic cells. In all cases in which pathologic changes were present in the parenchyma of the liver the vitamin A was markedly reduced. In nearly all cases in which the amount of characteristic fluorescence was slight, evidence of damage to the hepatic cells was present. Pathologic changes in the periportal areas did not exert a decisive effect on the distribution of the vitamin. Nutritional effects on the amount of vitamin A in the liver were observed only after prolonged starvation or the administration of large doses of vitamin concentrate. Popper, therefore, concludes that the functional state of the liver is more important than nutritional influences in determining the amount of vitamin A in that organ. He also emphasizes the importance of the Kupffer cells in the metabolism of vitamin A and the evidence that in some cases of hepatic damage a block in the exchange of vitamin A between the Kupffer cells and the parenchymal cells of the liver is present.

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100. Popper, H.: Histological Distribution of Vitamin A in Human Organs Under Normal and Under Pathologic Conditions, Arch. Path. **31**:766 (June) 1941. Meyer, K. A.; Popper, H., and Ragins, A. B.: Histologic Distribution of Vitamin A in Biopsy Specimens of the Liver, Arch. Surg. **43**:376 (Sept.) 1941.

101. Popper, H., and Ragins, A. B.: Histologic Demonstration of Vitamin A in Tumors, Arch. Path. **32**:258 (Aug.) 1941.

Editorial

TOXIC REACTIONS TO THE SULFANILAMIDE COMPOUNDS

Some of the most efficient therapeutic agents have undesirable side reactions even when judiciously employed. The sulfanilamide compounds are an example, although some reduction in the incidence of reactions has accompanied increased care in dosage and administration. Reactions following sulfanilamide therapy cannot apparently be altogether prevented. Now a group of investigators [1] claim to have devised a comparatively simple formula which is effective experimentally in reducing the toxic possibilities of sulfanilamide, sulfapyridine (2-[paraaminobenzenesulfonamido]-pyridine) and sulfathiazole (2-[paraaminobenzenesulfonamido]-thiazole) and yet does not result in a decrease in therapeutic efficacy.

The work which is reported in this issue of the ARCHIVES appears to be based on the assumption that detoxication in the body is obtained by oxidation, reduction and conjugation and that the principal compounds involved in this process are aminoacetic acid, choline, cystine, glucuronic acid, glutamine, sulfates, acetates and ornithine. Toxicity of sulfanilamide is ascribed to a sudden withdrawal of acetate precursors in the body during the process of conjugation by acetylation. Based on these concepts, the formula is intended to decrease the amount of acetylation by forcing conjugation into other channels, such as the formation of glucuronate.

Mice were used for toxicity studies, dogs for the determination of the rate of absorption and rats and rabbits for the measurement of the degree of conjugation. After trying the effects of various agents by oral administration, the authors concluded that calcium glucuronate was the most efficacious single compound and that a combination of aminoacetic acid cystine, calcium glucuronate and ascorbic acid provided "the most consistent and the greatest protection." Their experiments convinced the investigators that loss of therapeutic efficacy does not occur when such agents are simultaneously administered with a sulfanilamide compound.

Knowledge of the pharmacologic and clinical possibilities of sulfanilamide and its derivatives is undeniably incomplete; available reports on

1. Martin, G. J.; Fisher, C. V., and Thompson, M. R.: Therapeutic and Prophylactic Detoxication of Sulfanilamide, Sulfapyridine and Sulfathiazole, Arch. Int. Med., this issue, p. 662.

the prevention or diminution of toxic reactions following their use indicate that this aspect of study is even less complete. New communications based on animal or clinical investigations must therefore be critically considered from all points of view. Before the broad implications of this contribution are accepted, further substantiation under carefully controlled experimental conditions is obviously desirable.

News and Comment

The Herman M. Biggs Memorial Lecture.—Dr. James S. McLester, Professor of Medicine at the University of Alabama, Chairman of the Council on Foods and Nutrition of the American Medical Association and Chairman of the Subcommittee on Medical Nutrition of the National Research Council, delivered the Herman M. Biggs Memorial Lecture on Thursday, April 2, at Hosack Hall, the New York Academy of Medicine, under the auspices of the Committee on Public Health Relations.

His subject was "Nutrition and the Nation at War."

American Heart Association.—The eighteenth scientific meeting of the American Heart Association will be held June 5 and 6, 1942, at Chalfonte-Haddon Hall, Atlantic City, N. J.

CORRECTIONS

On page 278 of the review "Diseases of Nutrition" by Drs. Butt, Leary and Wilder in the February issue (ARCH. INT. MED. **69**:277, 1942) the manner of citation of a paper by Wolbach and Bessey (Vitamin A Deficiency and the Central Nervous System, Am. J. Path. **17**:586 [July] 1941) implied that these authors stated vitamin A deficiency causes overgrowth of skeletal tissues. Actually they reported that in vitamin A deficiency in rats skeletal growth is retarded earlier than growth of the soft tissues in general, including that of the central nervous system. They have shown that in the white rat at least the nervous manifestations are due to pressure effects caused by the relative overgrowth of the central nervous system.

In the article by Drs. Rosenberg, Dockerty and Meyerding entitled "Coccidioidal Arthritis: Report of a Case in Which the Ankles Were Involved and the Condition Was Unaffected by Sulfanilamide and Roentgen Therapy," in the February issue (ARCH. INT. MED. **69**:238, 1942), in the heading for table 1, on page 242, "Acute Granulomatous Coccidioidomycosis" should read "Chronic Granulomatous Phase of Coccidioidomycosis."

Book Reviews

Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases. By Richard P. Strong, M.D., Professor of Tropical Medicine, Emeritus, Harvard University. Sixth edition, 2 volumes. Price, $21. Pp. lix + 1,747, with 398 illustrations. Philadelphia: P. Blakiston's Son & Company, 1942.

In 1914 Admiral Stitt prepared a modest handbook on tropical diseases. It was only 421 pages long, was simply written and, as *The Journal of Tropical Medicine and Hygiene* reported (**18**:96 [April 15] 1915), was so planned that students could visualize the subject from every angle.

The book was deservedly popular, so that new editions were necessary every few years. The fifth edition was printed in 1929. It had kept pace with advancing knowledge and was 918 pages long. The author, however, succeeded in maintaining the book's character and charm. *The Journal of Tropical Medicine and Hygiene*, which had studied each edition carefully, was glad to point out that even though fifteen years old, Stitt's "Tropical Diseases" could still be recommended as a reliable guide to all members of the medical profession (**32**:172 [June 15] 1929).

The sixth edition is more elaborate than its predecessors and appears in two volumes; each is 850 pages long. The book has changed hands to a certain extent, for the first five editions represented the views of the United States Army, Navy and Public Health Service on tropical medicine, whereas the sixth edition emanates from the Department of Tropical Medicine of Harvard University.

Admiral Stitt and Dr. Strong are close friends of more than forty years' standing. One suspects that they have studied tropical medicine together, have seen eye to eye on many subjects and have watched the growth of knowledge in their favorite medical field with justifiable pride. Therefore, the new author of the sixth edition of Stitt's "Tropical Diseases" carries forward skilfully the same philosophy which made the earlier editions so successful and through his method of writing adds color to the blue and gold of the Navy by a dash of Harvard crimson without in any way hurting the original product.

The format of the two volumes is excellent. The illustrations are clear. More attention now is paid to current literature, so that at the end of each section has been added a trustworthy bibliography, which will appeal to the student. The arrangement of subject matter is much as it was in earlier editions. Diseases caused by protozoa, bacteria and filterable viruses are well described. There are interesting chapters on nutritional disorders of the tropics and on diseases caused by fungi, plants, animals or unrecognized agents. There is so much more to be told of prevention of disease in the tropics and of tropical hygiene than heretofore that the title of the book has been changed to lay emphasis on this fact.

It must be gratifying to two such old friends as are Admiral Stitt and Dr. Strong to have so fine a book evolve from their combined efforts. It must be even more gratifying for them to realize that this new edition was ready at a time when such a comprehensive manual would be seized on by, be studied by and be useful to vast numbers of those very men whom they always have found most congenial—the soldiers, sailors and public health officers whose job it is to meet medical problems under field conditions in all the warm corners of the earth.

Beiträge zur Röntgendiagnostik der Otitis Media Acuta und ihrer Komplikationen im Schläfenbein. By Sölve Welin, from the Diagnostic Division of the Otolaryngological Clinic of the University of Lund. Acta radiol., supp. XLII. Price, 20 kronor. Pp. 180, with 105 illustrations. Lund, Sweden: Håkan Ohlsson Boktryckeri, 1941.

This monograph is a contribution to the study of the importance of the clinical investigation of acute otitis media. Especially when the clinical findings are

indecisive, roentgen research often supplies the necessary data for determining whether there exists a pathologic change in the bone and whether this change is significant. This study is based on 926 patients seen between 1934 and 1939 whose conditions were given a diagnosis of otitis media. Of this group, 226 patients had bilateral acute inflammation of the middle ear, 338 had an inflammation only on the right side and in 362 the condition was confined to the left side.

Of especial practical value is the chapter on technic, which contains detailed instructions for the positions advocated by Henle, Schüller, Lange, Sonnenkalb, Mayer, Altschul, Stenvers and Runström. Still other positions might have been utilized, but these are the most informative. In each instance the positions are illustrated by lucid drawings and well reproduced roentgenograms. An excellent chapter deals with roentgen anatomy in children and adults. Other chapters discuss the roentgen pathology of indeterminate or obliterated intercellular structures, classified according to the lime content and all generously illustrated by typical cases. Special attention is given to labyrinthitis, perisinal abscess and apicitis, with all of which the roentgen findings are practically indispensable. An elaborate bibliography terminates the book.

Treatment of the Patient Past Fifty. By Ernst P. Boas. Price, $4. Pp. 324, with 19 illustrations. Chicago: The Year Book Publishers, Inc., 1941.

The reviewer has no criticism of this book, which is well written by a man who obviously knows what he is talking about; he does, however, have a considerable doubt as to the need of a separate book on the medical treatment of the aged. After all, no new principles are invoked, and one really deals with a purely artificial division if one separates people into those over and those under 50. One discerns in all this the lush tendency to overdo, to specialize artificially and in other ways to make medicine as cumbersome and complicated as possible which seems to have caught every one nowadays. Followed to its logical conclusion, this idea may in the end produce books on the medical treatment of adolescents, of young adults, of persons in the middle decades, etc., etc.—clearly a reduction to absurdity.

About Ourselves. By James G. Needham, Ph.D. Price, $3. Pp. 269, with illustrations. Lancaster, Pa.: Jaques Cattell Press, 1941.

This volume is what the author says it is—a survey of human nature from the zoologic viewpoint. It is an "exposition of human nature without any plans for its improvement."

In Part I—Man in His Biological Aspects—are discussed such matters as factors common to all living things, man's ancestry, development of behavior, instinct, learning and man in his environment.

Part II—Society in Its Biological Aspects—consists of a discussion of the components of social behavior, the components being physiologic activities, instincts, customs and reason. These component parts are also discussed in relation to war, government and religion.

The subject matter, though complex in nature, is simply given and is such that it will be comprehended and enjoyed by the reader, lay or professional.

Roentgen Treatment of Infections. By James F. Kelly and D. Arnold Dowell. Price, $6. Pp. 432, with 122 figures and 25 tables. Chicago: The Year Book Publishers, Inc., 1942.

The actual discussion of treatment of infections is preceded by chapters on roentgen rays and roentgen therapy in general which are concise and well written. Infection is discussed in great detail, and there is a good deal of material which is somewhat beside the subject. The writers are clearly enthusiastic supporters of radiation therapy of infections. However, their suggestion that it may be more useful than therapy with sulfanilamide or any of its derivatives (page 381) is

perhaps open to argument, and the enthusiasm of the writers for roentgen therapy of rheumatoid arthritis and various other conditions is not entirely supported by the reviewers experience.

Infant Nutrition. By Williams McKim Marriott and P. C. Jeans. Third Edition. Price, $5.50. Pp. 475, with 31 illustrations. St. Louis: C. V. Mosby Company, 1941.

The addition to knowledge of infant nutrition has been so great in the last few years that Dr. Jeans has appropriately taken on himself the duty of reediting this standard textbook. The chapters on growth and development and on metabolism are well written, and the new knowledge is brought out in fairly simple fashion, so that the text is not confusing to the medical student or the general practitioner, the readers to whom it would most appeal. The book answers satisfactorily many of the questions that mothers ask about their babies and tells what to do in the correction of many minor, but to the household in which a baby lives very important, ailments.

The Treatment of Burns. By Henry S. Harkins. Price, $6.50. Pp. 457, with 120 figures. Springfield, Ill.: Charles C. Thomas, Publisher, 1942.

The reviewer's first thought was how can there be enough to say about burns to fill such a large volume, but on going through the book one finds no superfluous material. The writer has achieved a monograph of the first order, scholarly, well arranged, dealing with every phase of the subject and documented by no less than thirteen hundred and twenty references. The work is richly illustrated by every conceivable sort of chart and diagram and many graphic pictures of clinical cases. In view of the war, this monograph seems especially timely.

Diabetes Mellitus. By Zolton T. Wirtschafter and Morton Korenberg. Price, $2.50. Pp. 186, with 9 figures and 3 tables. Baltimore: Williams & Wilkins Company, 1942.

This little book on diabetes is different from most similar productions, in that it deals almost altogether with the pathologic physiology of the disease. While many controversial questions are discussed, the position of the writer seems to be sound and the material is well documented with a bibliography of several hundred references. The book should be of great use in supplementing the ordinary clinical treatises.